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Precipitation of ultrafine particles using liquid antisolvent with concentric ultrasonic nozzle and subcritical CO2

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ABSTRACT

PRECIPITATION OF ULTRAFINE PARTICLES USING LIQUID ANTISOLVENT WITH CONCENTRIC ULTRASONIC NOZZLE AND SUBCRITICAL CO_2

by
Satya Chaitanya Kunchala

Nanotechnology has become more relevant in the pharmaceutical industry. Almost 40% of the drugs developed by pharmaceutical industries are poorly soluble in water which limits the bioavailability of these drugs in body fluids. Hence, it is necessary to reduce the particle size, which increases the interfacial surface area. This work focuses on micronization of class ii drug Griseofulvin which is poorly water soluble. Two processes namely, liquid antisolvent precipitation using ultrasonic nozzle and other process using subcritical CO_2 have been used to produce ultrafine particles of this drug.

In liquid antisolvent precipitation, a combination of ultrasound, polymer and surfactant is used to precipitate ultrafine particles. Ultrasound is used to increase the nucleation rate by enhancing the micro mixing of solvent and antisolvent stream and decrease the agglomeration. Surfactants and polymers are used to reduce the surface tension and thereby increase the nucleation rate. Use of additives can inhibit the particle growth and reduce the agglomeration. Particles in the range of 10 - 2 μm have been precipitated in this process.

The process using subcritical CO_2 has also been used to increase the micro mixing and to increase the nucleation rate. The depressurization of CO_2 expanded solutions of Griseofulvin, causes large temperature drop and hence induce low supersaturation and

high nucleation rate. This results in precipitation of ultrafine particles. Particles in the range of 15 - 1 μm have been precipitated in this process.

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by
Satya Chaitanya Kunchala

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APPROVAL PAGE

PRECIPITATION OF ULTRAFINE PARTICLES USING LIQUID ANTISOLVENT WITH CONCENTRIC ULTRASONIC NOZZLE AND SUBCRITICAL CO_2

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To my husband, Satish; my parents, Mr. Linga Murthy and Mrs. Usha Rani

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CHAPTER 1

INTRODUCTION

1.1 Nanotechnology

In recent years, particulate materials of sizes ranging from centimeters to nano meters have received considerable attention from science and industries such as materials and manufacturing, chemical and pharmaceutical, medicine and healthcare, environment and energy, biotechnology and agriculture, electronics, computation and information technology. As the particle size changes from centimeters to nano meters, material properties also change. Nanoparticles exhibit many special chemical, mechanical, optical, magnetic and electrical properties. Nanoparticles fall into three major groups [1].

- Natural nanoparticles – Naturally formed nano particles. Examples: volcanic ash, ocean spray, magnetotactic bacteria, mineral composites, and other existing particles in the environment.
- Incidental nanoparticles – By-products produced from industrial processes.
- Engineered nanoparticles – Specially designed particles to serve certain purpose. Example: fullerene C60 - used for fuel cell applications.

The process and technology, which purposely produces the materials, by manipulating their atoms, is called Nanotechnology. The main principle of nanotechnology is to build chemically stable structures that do not violate existing physical law [1]. It involves working with materials and devices that are at the nanoscale level. A nanometer is one billionth of a meter. That is, about 1/80,000 of the diameter of a human hair, or ten times the diameter of a hydrogen atom [2]. Scientists are very much interested in producing nano materials because these materials will have either enhanced or entirely new different properties from their parent particles. Nanoparticles drastically

change the bulk properties of the materials. According to material science models, composites made of particles of size smaller than 100nm, are much stronger than the ordinary composites made of particles with sizes greater than 100nm. These drastic changes stem from the weird world of quantum physics. Usually bulk properties of any material can be calculated by averaging the quantum forces affecting the atoms. As the size becomes smaller and smaller, there will be a point where the averaging does not works [3]. The properties of materials can be different at the nanoscale for two main reasons [3]

- Nanomaterials have a relatively larger surface area when compared to the same mass of material produced in a larger form. This can make materials more chemically reactive (in some cases materials that are inert in their larger form are reactive when produced in their nanoscale form), and affect their strength or electrical properties.

- Quantum effects can begin to dominate the behavior of matter at the nanoscale particularly at the lower end, affecting the optical, electrical and magnetic behavior of materials. At nanoscale, materials can be produced in one dimension such as, thin surface coatings, in two dimensions such as, nanowires and nanotubes and in three dimensions such as, nanoparticles.

1.2 Applications of Nanotechnology

Nanotechnology is the revolutionary science and art of manipulating the matter at atomic or molecular scale that has cut across such disciplines as chemistry, physics, biology, and engineering [4]. The products of advanced nanotechnology that will become available in coming decades promise even more revolutionary applications than the products of current and near-term nanotechnology. Applications in medicine and environment are very important to discuss.

1.2.1 Applications in Medicine

Nanomaterials have received grater interest in the pharmaceutical field. Due to advances in nano technology, effective delivery of pharmaceuticals is possible with nanoparticles. In drug delivery pharmacokinetics and pharmacodynamics plays a vital role. Pharmacokinetics deals with drug delivery inside the human body. When drug administered intravenously, it goes through a number of stages such as absorption, distribution, metabolism & elimination. Drug efficacy mainly depends on the physiochemical properties of the drug (molecular weight, charge, and aqueous solubility) and therefore on its chemical structure. Main purpose of many drug delivery systems is targeted drug delivery. A successful drug delivery system should demonstrate the properties like optimal drug loading and release, longer shelf life and lower toxicity. Nanoparticles are desirable for drug delivery because of many important properties like high drug solubility, lower toxicity, bioavailability and targeted drug delivery [5]. Nanomachines can deliver drugs to targeted sites within a patient's body. Specific nanomachines can remove obstacles in the circulatory system and used in identifying and killing the tumor cells [2].

Table 1.1 Applications of Nanotechnology in Medicine [6]

Structure	Size	Role in drug delivery
Carbon magnetic Nanoparticles	40-50 nm	For drug delivery and targeted cell destruction
Dendrimers	1-20 nm	Holding therapeutics substances such as DNA in their cavities
Ceramics Nanoparticles	~35 nm	Accumulate exclusively in the tumor tissue and allow the drug to act as sensitizer for photodynamics therapy without being released
Chitosan Nanoparticles	110-180 nm	High encapsulation efficiency .In vitro release studies show a burst effect flowed by a slow and continuous release.
Liposomes	20-25 nm	A new generation of liposomes that incorporate fullerenes to deliver drug that are not water soluble, that tend to have large molecules
Low Density Lipoprotein	20-25 nm	Drug solublized in the lipid core or attached to the surface
Nanoemulsions	20-25 nm	Drug in oil/or in liquid phases to improve absorption
Nanolipispheres	25-50 nm	Carrier incorporation of lipophilic and hydrophilic drugs
Nanoparticles composites	~40 nm	Attached to guiding molecules such as Mabs for targeted drug delivery
Nanoparticles	25-200 nm	Act as continuous matrices containing dispersed or dissolved drug
Nanopill/Micelle	20-45 nm	Made for two polymer molecules-one water - repellent and the other hydrophobic-that self assemble into a sphere called a micelle that can deliver drugs to specific structures within the cell
Nanospheres	50-500 nm	Hollow ceramic nanospheres created by ultrasound
Nanovesicles	25-3000 nm	Single or multilamellar bilayer spheres containing the drugs in lipids
Polymer Nanocapsules	50-200 nm	Used for enclosing drugs

Nanodevices, such as the respirocyte (artificial red blood cell) can replace defective or improperly functioning cells. Applications of these respirocytes include transfusable blood substitution, partial treatment of anemia, prenatal/neonatal problems, and lung disorders [7]. Other applications of nanotechnology are nanorobots. In human body, nanorobots can monitor the levels of different compounds and record the information in the internal memory. In a given tissue, nanorobots in detail can examine biochemical, biomechanical and histometrical features [2].

1.2.2 Environmental Applications

Nanotechnology offers new and improved environmental applications. However, this technology has some problems, such as new toxins and related environmental hazards, associated with it. Nanotechnology is playing an important role in pollution reduction. Home lighting based on this technology could reduce energy consumption by 10% in the United States, saving $100 billion annually and reducing carbon emissions by 200 million tons per year [8]. Nanostructured catalysts can make chemical manufacturing more efficient by providing higher selectivity for desired reaction products [9]. Nanotechnology applications can be helpful in creating the substances that replace currently used toxic materials. For example, nontoxic, energy-efficient computer monitors are replacing those made of cathode ray tubes (CRT), which contain many toxic materials [10].

Nanotechnology plays an important role in remediation and end-of-pipe treatment technologies. Varieties of nanoparticles are used for the treatment and remediation of pollutants in the environment. Nanoparticles can also exhibit unique chemical reactivity which is not observed in larger particles. Scientists are more interested in manipulating the surface of nanoparticles with organic or inorganic dyes in order to extend their photo response from UV to visible light, to make them more efficient as photo catalysts for the transformation of environmental contaminants [11]. An example of environmental treatment and remediation-related application of nanomaterials includes using nanostructures for polymer-supported ultra filtration (PSUF) [12].

Rapid and precise sensors made of nanoparticles are capable of detecting pollutants at the molecular level. These are greatly helpful to protect human health and the environment. Nanotube sensors exhibit fast responses at room temperature to the

gases and these are more sensitive than existing solid-state sensors [13]. Human health and ecological diagnosis could also benefit from sensors. Nanobarcodes can greatly enhance our ability to identify the source and strength of contaminants, determine the route and mechanism of environmental fate and bioavailability, and assess the effectiveness of treatment and remediation techniques [14]. Manufacturing process control, compliance, ecosystem monitoring, and environmental decision-making would be significantly improved if more sensitive and less costly techniques for contaminant detection were available [13].

Nanotechnology is a revolutionary scientific and engineering venture that will invariably impact the existing infrastructure of consumer goods, manufacturing methods, and materials usage. Not surprisingly, the potential benefits have dominated scientific and mass media coverage of nanotechnology. But any technology can be a double-edged sword. We are already witnessing some precursors of nanotechnology-associated pollution. Toxic gallium arsenide used in microchips enters landfills in increasing quantities as millions of computers and cellular phones are disposed of every year. Potentially harmful effects of nanotechnology might arise as a result of the nature of nanomaterials themselves, the characteristics of the products made from them, or the aspects of the manufacturing process involved. The large surface area, crystalline structure, and reactivity of some nanoparticles, for instance, may facilitate transport of toxic materials in the environment, or the size and chemical composition of nanostructures may lead to biological harm because of the way they interact with cellular materials [15]. Because nanotechnology is unlikely to be an entirely benign technology

advance, there is an urgent need to evaluate the effectiveness of current water and air treatment techniques for the removal and control of potential nanoscale pollution.

However, people must remain mindful of the potential ramifications of this technology, including the fact that nanoscale materials can enter the food chain and be absorbed or transported by water and food. Bioavailability and toxicity of newly created nanoscale materials are largely unknown. Nanotechnology is highly interdisciplinary and may present further challenges for environmental scientists and engineers.

1.3 Problem Definition

From the reports of pharmaceutical industry, it is said that, around 40% of the drugs being developed by the industries are not soluble in water [16, 17]. Hence, these drugs have poor wetting properties and less ability to dissolve with gastrointestinal fluids. It is a great challenge for the scientists to improve dissolution properties of the drug in order to improve the bioavailability. According to the Noyes-Whitney equation, as the particle size decreases, its surface area increases and thus improves the dissolution properties [18]. There are many commercially used methods to reduce the particle size such as mechanical comminution (crushing, milling, grinding, and jet milling), thermal recrystallization, spray drying, freeze drying and recrystallization using solvent evaporation etc. Techniques described above are associated with many drawbacks. Mechanical comminution is simple and economical, but it is not able to accomplish desired amount of particle size reduction and it is not suitable for thermally sensitive compounds. Thermal recrystallization, spray drying methods requires using lot of solvents. In addition, all these methods requires relatively high-energy and yet suffer

from poor control in maintaining particle size, PSD and morphology [19]. Though Liquid Anti solvent method is simple and economical technique for pharmaceutical industry, there are some drawbacks such as uncontrolled growth and agglomeration of nano particles after the formation and particle growth by Ostwald ripening. These limitations affect the quality and stability of the drug. Nanoparticles can provide required surface area improvement. However, production of particles at nanoscale ranges remains challenging [20, 21, 22].

1.4 Objectives

This research project focuses on production of ultrafine particles of Griseofulvin through Liquid Anti solvent crystallization by using ultrasound nozzle and by using CO_2. Problems associated with Liquid Antisolvent process can easily overcome by using ultrasound concentric nozzle. Ultrasound nozzle increases the nucleation rate and decreases the particle agglomeration. Due to atomization created by the nozzle, both solvent and antisolvent can be sprayed in the form of fine droplets. As a result, fine particles can be precipitated. Use of surfactants and polymers can control the particle size. Surfactants and polymers, reduce the surfacetension thereby increases the nucleation rate. The process that uses CO_2 can also produce ultrafine particles. When solution containing CO_2 is depressurized, CO_2 evaporates from solution at normal atmospheric pressure. Removal of latent heat from the solution reduces the solution temperature causes supersaturation in solution. Supersaturation induces nucleation and initiates precipitation process. Thus fine particles can be produced.

1.5 Organization of Thesis

In this thesis after the Introduction, Chapter 2 presents a review on production of Ultrafine particles. Chapter 3 focuses on the selected methods used to produce nano particles and materials used. Chapter 4 presents results and brief discussion on the experimental work done. Chapter 5 presents conclusions based on the research and suggestions for future work. Chapter 6 includes references.

CHAPTER 2

PROCESS FOR PRODUCTION OF ULTRAFINE PARTICLES-AN OVERVIEW

Over the decades, many particle production processes have been developed for use in pharmaceutical industries. To enhance the properties of poor water soluble drugs, nanoparticle production processes such as, mechanical micronization techniques, supercritical fluid techniques, cryogenic spray processes, and solvent evaporation processes have been developed.

2.1 Mechanical Micronization Techniques

Grinding and high pressure homogenization techniques are commercially popular methods for reducing particle size. Dry grinding with media mill and jet mill limits the particle size to few microns. Hence, wet grinding with ball media mill and liquid jet mill came in to picture in late 1990's. There is some equilibrium between the particle size and grinding time. Once the particle reaches its minimum size, with the increase in grinding time particle size starts increasing due to particle agglomeration [67]. Homogenization process uses high pressure to break down the particles in the suspension.

2.1.1 Wet Grinding

Grinding is an attrition process. In this process, grinding media moves against one another and against the walls of the mill. Grinding at lower speeds gives fine particles but longer milling times are required. Finer particles can be produced by using wet milling techniques [23]. In the wet milling process, the poor water-soluble drug is dispersed in an aqueous-based surfactant solution, and then the resulting suspension is wet milled with

the grinding media. The amount of grinding media, stabilizer and the raw material used may vary with the type of mill. These are the main important factors affecting the efficiency of the mill and particle fineness. Usually grinding media fills about 30% to 50% of the volume of the mill chamber [23]. High-energy-generated shear forces and the forces generated during impaction of the milling media with the solid drug provide the energy to crush the drug particles into nanometer size [21, 24]. Main limitation of the wet milling process is contamination of the drug by grinding media used [25]. During the milling process, due to erosion of grinding materials product is contaminated. In addition to that, wet milling is a batch process. There is batch-to-batch variation detected in the quality of dispersion, processing times, drug crystallinity and particle size distribution. Due to these variations, drug particle stability, powder flow properties, and efficiency of delivery system are affecting. In some cases, milling over the days brings the risk of microbiological problems, especially when performing the milling at lower temperature (temperatures below 30 °C) or having dispersion media providing nutrition to bacteria.

2.1.2 High Pressure Homogenization

High pressure homogenization is the other mechanical micronization process for reducing the particle size of poor water soluble drugs present in liquid suspensions. With this method it is possible to make more stable suspensions for enhanced clinical effectiveness. As the active ingredients are homogeneously distributed in the suspension, bioavailability of the drug increases. In this method drug is dispersed in an aqueous solution by high speed stirring to get a suspension. Obtained suspension is then passed through a high pressure homogenizer through 3 to 20 cycles at a pressure of 1500 bar [25]. Then at the same pressure, suspension passes through a very small homogenization gap having a

width of 25 μm. Due to its small width, dynamic fluid pressure increases with increase in streaming velocity of the suspension. As a result, water starts boiling at room temperature and cavitation occurs when fluid leaves the homogenization gap [25]. These cavitation forces are strong enough to break the drug microparticles into drug nanoparticles [25, 26]. Particles produced from this process have an average size of 40 nm to 500 nm, and particles greater than 5 μm are less than 0.1% in the total population [25, 26]. The particle size depends on the hardness of the drug substance, processing pressure and number of cycles applied. Size can be controlled by adjusting the operating parameters like pressure and cycles applied. Application of high pressures tends to give more amorphous particles. The stabilization against aggregation and coalescence is the main challenge while forming nanosuspensions. Stability of nanosuspensions can be determined by the zeta potential. In addition to that, Ostwald ripening also determines the stability of highly dispersed systems [25]. The absence of Ostwald ripening indicates the long-term physical stability of an aqueous suspension [27]. It was observed that there was no Ostwald ripening in the nanosuspensions produced by high-pressure homogenization process. Nano suspensions produced from this processes have less concentration differences, which is a main prerequisite for commercial large-scale production from this process. High-pressure homogenizers are available with different capacities from a few hundreds to a few thousands liters per hour [25, 26]. One disadvantage of this process is that, application of high pressures used can change particle crystallinity and batch-to-batch variation in crystallinity level might be an issue for quality control. Stability of partially amorphous nanosuspensions is the main challenge in pharmaceutical industry applications.

2.2 Supercritical Fluid [SCF] Technology

Since there are many disadvantages with high energy milling such as contamination and batch variation etc, another alternative method to produce nano particles is, Supercritical fluid technology. Supercritical fluids have higher viscosities and diffusivity coefficients and are good solvents. Physical properties of Supercritical fluids (SCF) such as liquid - like density, gas like diffusivity, negligible surface tension and liquid like viscosity can be manipulated over a wide range by varying both temperature and pressure. There are many advantages in selecting carbon dioxide from many Supercritical fluids as it has low critical Temperature (Tc) = 31.1°C and moderate critical pressure (Pc) = 73.8 bar, and it is nonflammable, nontoxic, and readily available in high purity. The basic principle of Supercritical fluid technology is precipitation of micron and sub micron particles by rapid depressurization of saturated solutions. Different SCF techniques such as Rapid Expansion of Supercritical Solutions (RESS) [28 - 33], Supercritical Antisolvent (SAS) [34, 35] and Depressurization of Expanded Liquid Organic Solutions (DELOS) [36 - 38] etc. are being investigated.

2.2.1 Rapid Expansion of Supercritical Solutions (RESS)

In this process drug is dissolved in supercritical fluid which acts as a solvent. This solution is depressurized through a nozzle, which has small orifice. The rapid expansion of supercritical fluid solutions through the small orifice produces an abrupt decrease in solubility of the solvent as it goes from a supercritical fluid state to a very low-density gas phase after the expansion. This results in the nucleation of any low-vapor-pressure solute species that were present in the solution prior to expansion. Controlled particle

size distribution and morphology mainly depends on the solubility of drug in supercritical fluid, operating parameters (temperature and pressure), nozzle geometry and dimensions.

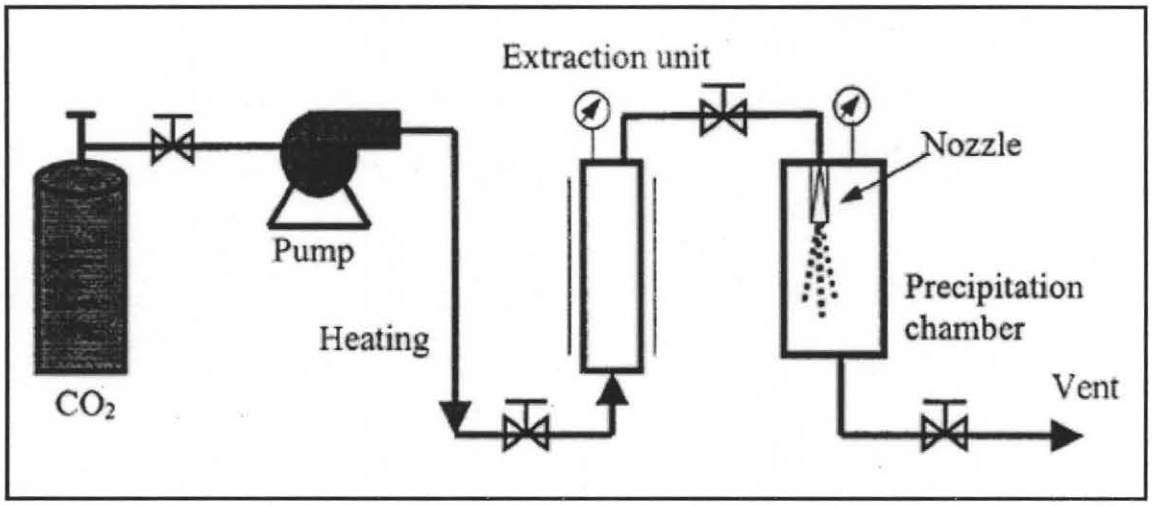


Figure 2.1 Schematic diagram of RESS process. [67]

A solid co-solvent can be added to increase the solubility of drug, which has low solubility in supercritical fluid. This process can be called as RESS-SC (Rapid Expansion of Supercritical Solutions – Solid Co-solvent). Later a solid co-solvent can be separated from the drug by using different techniques like lyophilization etc. The main advantage of this process is that it reduces the need for using harmful organic solvents. Though this process is simple and easy to develop, there are many limitations like poor solubility of many drugs in supercritical fluids, design of nozzle and scale-up problems involved in this method [28 – 33].

2.2.2 Supercritical Antisolvent (SAS) Precipitation

In this process drug is dissolved in an organic solvent and this solution is sprayed through a nozzle in to a high-pressure chamber. This chamber is filled with supercritical CO_2, which acts as antisolvent. When the sprayed solution comes in contact with supercritical CO_2, nucleation occurs by two way diffusion between supercritical CO_2 and the solution. Controlled particle size distribution and morphology depends on operating parameters (temperature and pressure) and nozzle dimensions. [34, 35]

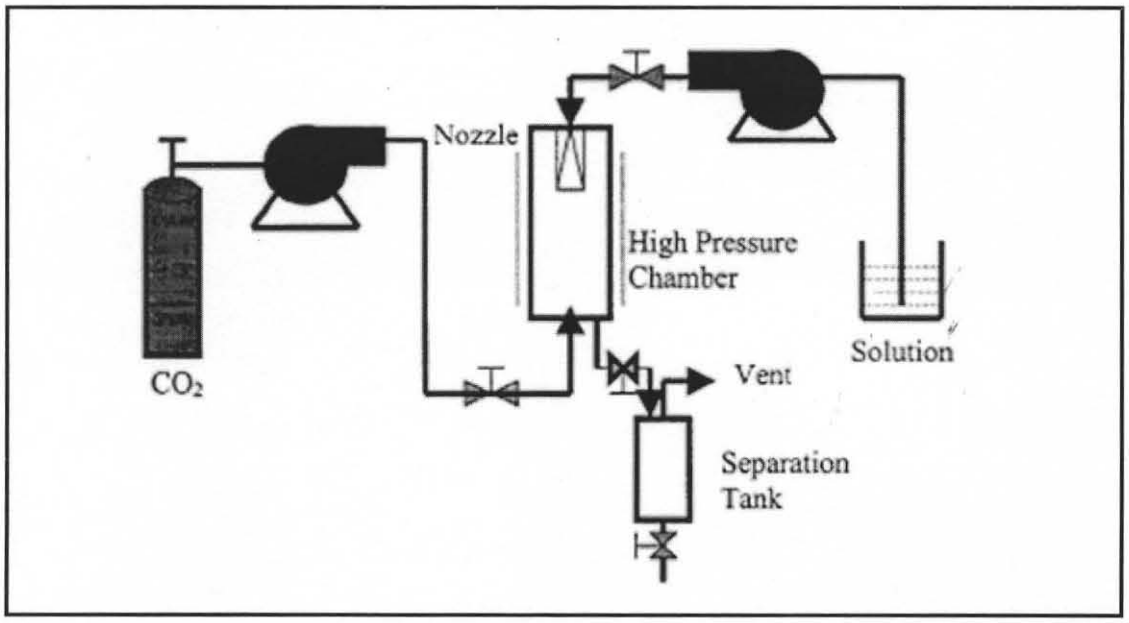


Figure 2.2 Schematic diagram of SAS process.[67]

2.2.3 Depressurization of Expanded Liquid Organic Solutions (DELOS)

In depressurization of Expanded Liquid Organic Solution (DELOS), super critical CO_2 acts as a co solvent for the formation of nano sized particles. The driving force behind DELOS process is a fast and large temperature drop. This occurs when the pressurized

solution is expanded from a working pressure (P_w) to atmospheric pressure. This fast drop in temperature causes the saturation limit to drop equally as fast causing the crystallization of particles from the solution. In DELOS process, solute is dissolved in an organic solvent in a pressure resistant chamber that is heated to a desired working temperature. Once this is complete, SC CO_2 is pumped to dissolve into the solution and used to achieve the desired working pressure (P_w). Sufficient time is provided for the ternary solution to reach equilibrium and the working temperature (T_w). Once equilibrium is achieved, the solution is expanded through a one-way valve into a chamber at atmospheric pressure. Pure nitrogen is pumped into the solution chamber to maintain the working pressure during expansion. A filter at the bottom of the expansion chamber collects the solute powder [37, 39].

It has been shown that the DELOS process is not dependent on the pressure change from the working pressure to atmospheric pressure. Ventosa, et al. [39] have shown that for a given system yield, particle size, and particle size distribution are dependent on the temperature drop from the working temperature to the final depressurization temperature (T_F), therefore the main factors that control yield are the working concentration of C_F and the initial solubility ratio [39]. This allows the process to be carried out at lower temperatures without any effects [39]. Since crystallization through the (DELOS) process is dependent on a large temperature drop, the yield can be maximized by maximizing the amount of SC CO_2 used.

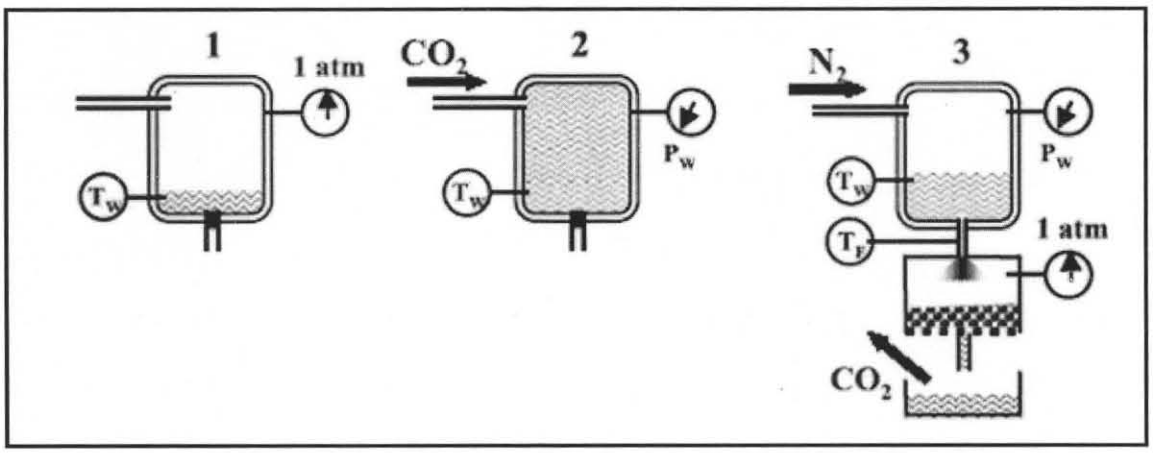


Figure 2.3 Schematic diagram of DELOS process.[39]

The main advantage of (DELOS) is encapsulation. It is very cost effective and nano powders formed from this process are of highest quality. The disadvantage is, solubility is limited and reaches saturation very fast and the particles formed are not in spherical shape and needs to be treated by other techniques to produce spherical shapes, which are costly [37, 39].

2.3 Spray Process

Another method to produce micro and nano particles is cryogenic spray process. Spray-freezing into vapor processes have been developed under cryogenic spray process. Usually, in these processes, halocarbon refrigerants and liquid nitrogen have been used as cryogenic media [40 - 51]. Generally, in these processes, drug solution is atomized through the nozzle in a chamber that contains cryogenic media. Atomized droplets gradually solidify and then freeze while they pass through the cryogenic media. A spray-freezing into vapor process has been developed by Gombotz *et al* and Gusman and Johnson [52 – 55]. In this process, atomization of drug solution takes place in nitrogen vapor. Solution

droplets gradually solidify while passing through nitrogen vapor and freeze when they come in to contact with cryogenic liquid [20]. There are some drawbacks like broad particle size distribution and presence of non-micronized particles in this process. To overcome this, a new cryogenic spray process has been developed and patented by The University of Texas at Austin in 2001 [56] and commercialized by The Dow Chemical Company.

2.3.1 Spray Freezing into Liquid (SFL) Process

In this process, a solution that contains a drug is atomized through a nozzle in to a compressed liquid. The compressed liquids used in this process are, compressed CO_2, helium, propane, ethane, or the cryogenic liquids including nitrogen, argon, or hydrofluoroethers [56]. Nano particle formation is due to intense atomization of drug solution and then rapid freezing in the cryogenic liquid medium. High degree of atomization occurs by spraying drug solution directly in to cryogenic liquid and rapid freezing is achieved by low temperature provided by cryogenic liquids and formation of high surface area droplets. The high degree of atomization and rapid freezing rates led to formation of amorphous nanostructured particles with high surface areas, enhanced wetting and significantly enhanced dissolution rates [20, 57, 58 - 64]. According to the need, obtained nano particles in the liquid medium are dried in lyophilizer to get dry powder. Powders produced from SFL process exhibits higher dissolution rates compared to the micronized bulk drugs. Powders produced from this drug can be used for different delivery systems such as respiratory delivery, oral delivery etc.

2.4 Solvent Evaporation Process

Two solvent evaporation techniques used for production of nano particles are described below. In both techniques, drug is dissolved in a solvent and then the solution is sprayed through the nozzle into a chamber. Nanoparticles are formed by the evaporation of solvent.

2.4.1 Spray Drying

Spray drying is a common method used in many pharmaceutical industries. In this method, typically, drug solution is pumped through a nozzle and is atomized in a chamber containing a hot gas. Production of Nano particles of sensitive materials requires oxygen free drying and hence nitrogen gas is used instead of hot gas. Different types of atomizers are used for spray drying such as, single fluid, two-fluid, and ultra-sonic designs. These different styles have different advantages and disadvantages depending on the application of the spray drying required.

Spray drying process is used by food and pharmaceutical industries as an encapsulation technique. Dehydrated powders such as instant drink mixes are produced by this technique. This technique is mainly used in food industries for the preparation of dehydrated milk. As this process causes thermal degradation, other techniques are used for the milk dehydration. Skim milk powder is still widely produced using spray drying technology around the world, typically at high solid concentration for maximum drying efficiency. Thermal degradation of products can be overcome by using lower operating temperatures and larger chamber sizes for increased residence times.

Recent research is now suggesting that the use of spray-drying techniques may be an alternative method for crystallization of amorphous powders during the drying process

since the temperature effects on the amorphous powders may be significant depending on drying residence times.

2.4.2 Evaporative Precipitation in to Aqueous Solution (EPAS)

This process was developed and patented by The University of Texas at Austin and licensed to The Dow Chemical Company in 2001. In this process, drug is dissolved in an organic solvent and is sprayed through a nozzle at high pressures and temperatures. Usually temperatures maintained are above the boiling points of the organic solvent and nozzle is placed in a heated aqueous solution. Nozzle is immersed in aqueous solution to avoid the crystal growth. According to need, stabilizers are added to organic solution and aqueous solution. Drug nano particle suspension is dried by using lyophilizer or spray dryer. There are many advantages with EPAS process over spray drying process. In EPAS Nanoparticles are formed due to rapid evaporation of the heated organic solution and fast nucleation. Varieties of hydrophilic stabilizers are found in order to control the particle size. These stabilizers work by diffusing to the surface of growing particles rapidly enough in order to prevent the particle growth.

When hydrophilic surfactant is used, it migrates towards the drug-water interface during particle formation, and the hydrophilic segment is oriented outwards towards the aqueous continuous phase. The stabilizer inhibits crystallization of the growing particles [65]. In spray drying process, this type of hydrophilic surfactant coating is not possible because of absence of water. Even after the solution is dried to form powder, hydrophilic surfactant is surrounded by drug. As a result of this dissolution rate of the drug increases. To prevent the agglomeration and particle growth, high surfactant adsorption levels on the surface of the particle are required. Applications of EPAS suspensions include, use in

parenteral formulations to enhance the bioavailability and in oral dosage forms. The ability to engineer stable particles with high potencies and high dissolution rates with EPAS presents new opportunities in the development of commercial formulations for poor water-soluble drugs.

2.5 Comments

In summary, recent advances in commercially or potentially commercially available nanoparticle engineering processes have been discussed. These nanoparticle engineering processes including wet milling, high pressure homogenization, RESS, SAS, DELOS, SFL, Spray drying and EPAS techniques have successfully incorporated poorly water soluble drugs alone, or with excipients into the microparticles or nanoparticles with significantly improved dissolution rates and bioavailability. As the percentage of poor water-soluble experimental compounds is increasing, nanoparticle-engineering processes for enhancement of dissolution rates of poorly water-soluble drugs offer great promise for pharmaceutical development and manufacturing to bring these experimental compounds into the market.

Though there are many processes available in the literature, precipitation of ultrafine fine particles of active pharmaceutical ingredients with low water solubility is still a difficult task. Inspite of all the available processes, Liquid antisolvent (LAS) is still a commercially viable and economically attractive process. In current research work, Liquid antisolvent process is used to produce ultrafine particles.

2.6 Liquid Antisolvent (LAS)

Liquid antisolvent techniques have been using in many industries to crystallize solid compounds. As this technique eliminates the use of thermal energy which can lead to the degradation of biological activity of drug particles, it has become one of the most promising particle production techniques in the pharmaceutical industry. In the most common procedures, a poor solvent of a particular drug is added to the drug solution in order to precipitate the solute. Water is most commonly used as an antisolvent for hydrophobic drug compounds, whereas organic solvents are used for hydrophilic compounds. Drug is precipitated upon mixing of solution and antisolvent. The working principle behind this technique is that during mixing, there is an increase in the molar volume of the solution that results in decrease in solubility power of the drug and hence the precipitation. Even though it has been widely used in industry, there is minimal control over the crystal morphology and size distribution. A combination of surfactants and ultrasound is used in order to control the particle size and distribution.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Drug

The drug Griseofulvin with molecular formula $C_{17}H_{17}O_6$ is an antifungal antibiotic. It is derived from a species of Penicillium. It is used in the treatment of ringworm and other fungal infections of the skin or nails.

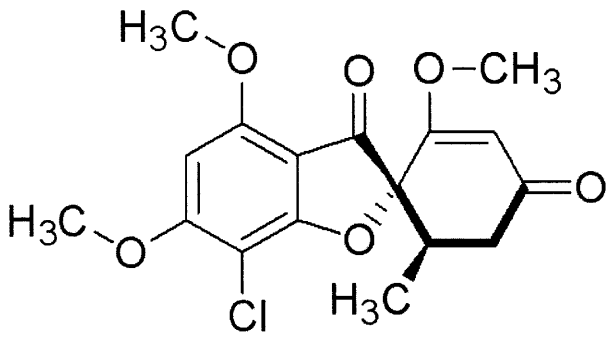
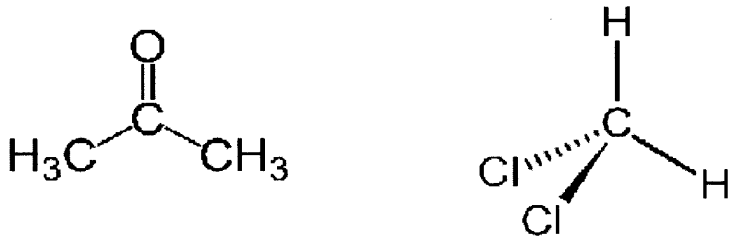


Figure 3.1 Griseofulvin structure.

Some research shows that it can be used to treat cancer as it inhibits the cell division in cancer cells. It does not interfere with cell division in healthy cells. ≥ 95% (HPLC grade) is purchased from Sigma Aldrich and is used without any further purification. Griseofulvin is poorly soluble in water but is soluble in many organic solvents such as acetone, DCM, DMF, ethanol and Triacetin.

3.1.2 Solvents

Solvents Acetone (CHROMASOLV®, for HPLC, ≥ 99.8%) and DCM (CHROMASOLV®, for HPLC, ≥ 99.9%) were purchased from Sigma Aldrich and were used without any further purification.



Acetone DCM

Figure 3.2 Acetone and DCM.

Acetone is an organic solvent with the formula CH_3COCH_3 and is miscible with water. Dichloromethane (DCM) is the chemical compound with the formula CH_2Cl_2. It is a colorless, volatile liquid widely used as a solvent for many chemical processes. It is miscible with most organic solvents and immiscible with water. Griseofulvin solubility in acetone is 38 mg/ml and in DCM is 0.220 gm/ml.

3.1.3 Surfactants

Surfactants Tween 80 (viscous liquid with average molecular weight 1310 and the composition of Oleic acid and ~70% (balance primarily linoleic, palmitic, and stearic acids)), Sodium Dodecyl Sulphate (Sigma Ultra, ≥99.0% (GC)) and Poloxamer 188 (a solid contains 100 ppm BHT with average molecular weight 8350) are purchased from Sigma Aldrich and are used without any further purification.

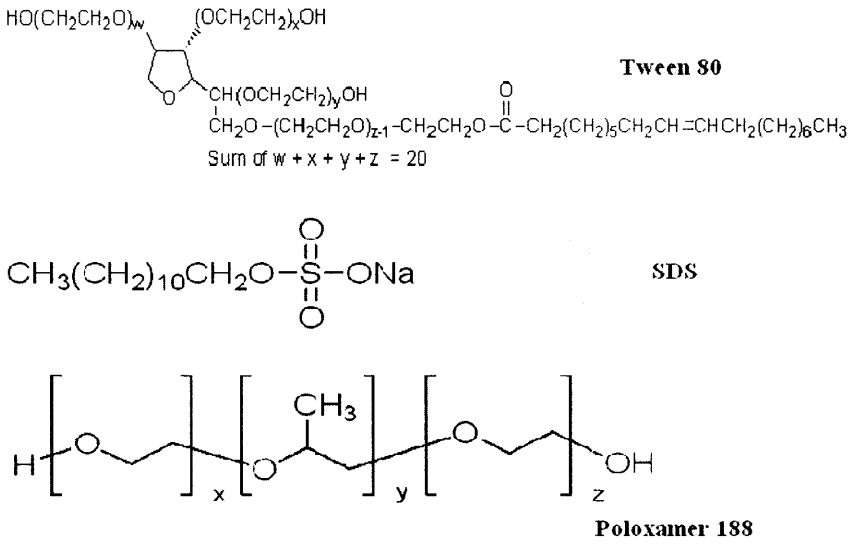


Figure 3.3 Tween-80, SDS, Poloxamer 188.

Tween 80 is a nonionic surfactant and emulsifier often used in ice cream to prevent milk proteins from completely coating the fat droplets. It is also known as Polysorbate 80. It is a viscous, water-soluble yellow liquid. Tween 80 can be used as an emulsifier in the manufacture of medications for parenteral administration. Its CMC is around 1.2* 10-5 M at 25°C.

Sodium Dodecyl Sulphate is an anionic surfactant with molecular formula $C_{12}H_{25}SO_4Na$ is used in household products such as toothpastes, shampoos, shaving foams, some dissolvable aspirins, fiber therapy caplets etc. The molecule has a tail of 12 carbon atoms, attached to a sulfate group, giving the molecule the amphiphilic properties required of a detergent. It is a smaller molecule as compared to Tween 80 and its CMC is around 0.0082 M at 25°C.

Poloxamer is a triblock copolymer. It has two hydrophilic polyethylene oxide ends and one hydrophobic polypropylene oxide segment in the middle. They can be used to increase the water solubility of hydrophobic, oily substances or otherwise increase the

miscibility of two substances with different hydrophobicities. For this reason, these are commonly used in industrial applications, cosmetics, and pharmaceuticals. They have also been used as model systems for drug delivery applications. Its CMC is 0.0004 M at 25°C.

3.1.4 Polymers

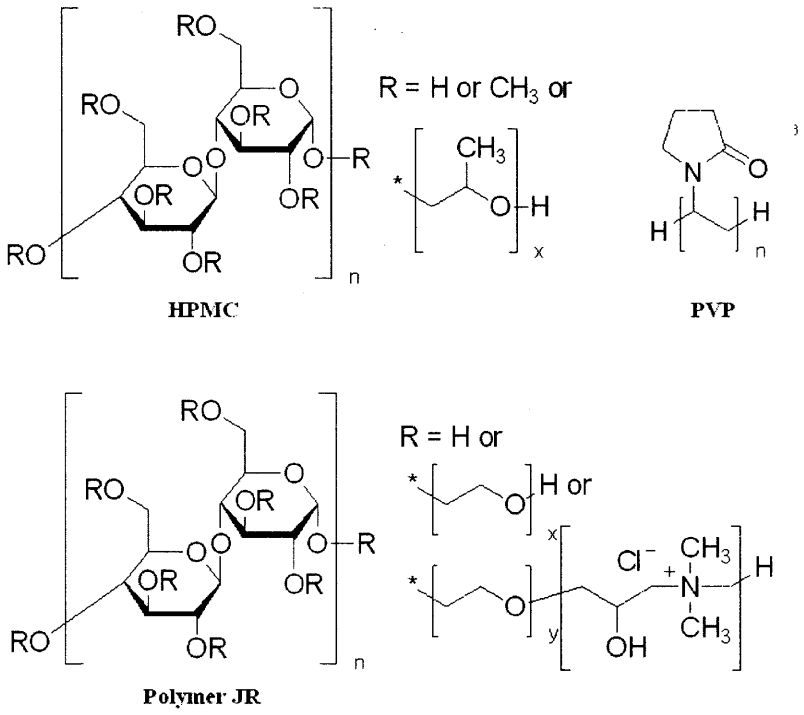


Figure 3.4 HPMC, PVP, Polymer JR.

Polymers HPMC, PVP (average mol wt 360,000) and Polymer JR are purchased from Sigma Aldrich and are used without any further purification.

Hydroxy Propyl Methyl Cellulose (HPMC) is a semisynthetic, inert, viscoelastic and non - ionic water-soluble polymer. It is used as an ophthalmic lubricant, excipient

and controlled-delivery component in oral medicaments. In oral tablet and capsule formulations, it is used to delay the release of a medicinal compound into the digestive tract.

PVP is a non ionic polymer and is soluble in water as well as other polar solvents. It is as an inhibitor of recrystallization and increases the solubility of drugs in liquid and semi-liquid dosage forms (syrups, soft gelatin capsules etc). PVP in its pure form can be edible by humans. Main applications include binder in many pharmaceutical tablets, stabilizer in food additives, used in personal care products such as shampoos and toothpastes, in paints, and adhesives like old-style postage stamps and envelopes etc. PVP added to Iodine forms a complex (Povidone - iodine) that possesses disinfectant properties. This complex is contained in various products like solutions, ointment, liquid soaps and surgical scrubs.

3.2 Methods

3.2.1 Experimental Methods

Two methods, Liquid antisolvent precipitation by using ultrasonic nozzle and MDELOS, are developed for the production of nano particles.

3.2.1.1 Liquid Antisolvent Precipitation using Ultrasonic Nozzle. In many pharmaceutical industries, liquid antisolvent technique is used for the production of nano particles. Since many of the drugs used in the pharmaceutical industry are thermally sensitive and poorly water-soluble, this technique is more suitable for the production of nano particles. In this technique typically, a poor solvent of a particular solute is added to the solution in order to precipitate the solute. When the solution and antisolvent are

mixed together, precipitation occurs. The most common antisolvent used for this technique is water when poorly water-soluble drugs are being precipitated. The working principle behind this technique is that during mixing, there is an increase in the molar volume of the solution that results in decrease in solubility power of the solute and hence the precipitation. Though these techniques are widely used in industry, these are also associated with some drawbacks such as, controlled particle size distribution and morphology.

Liquid antisolvent precipitation using ultrasonic nozzle involves three steps for the production of nano particles. Figure 3.5 schematically shows the steps involved.

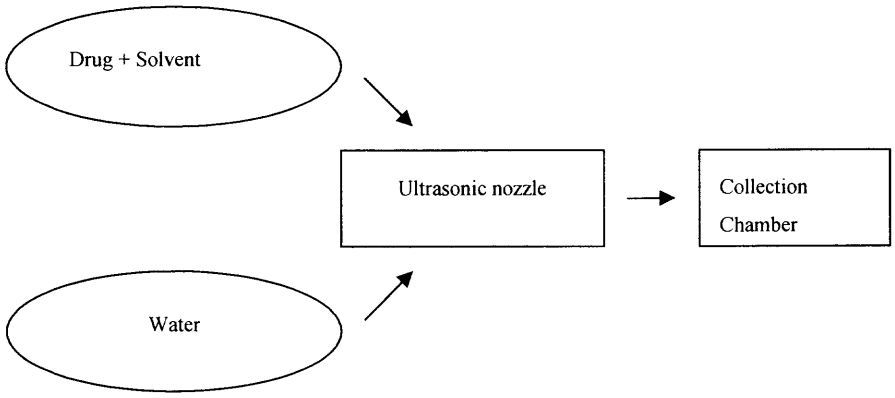


Figure 3.5 Flow diagram of Liquid anti solvent process with ultrasonic nozzle.

Solution of a drug in the solvent is prepared. The power of ultrasound unit applied to the nozzle is set in order to atomize the solution coming from pumps. Atomized solution is directly collected in a chamber containing water with stabilizers. The concentric ultrasonic nozzle consists of inner nozzle with ID of the nozzle is 0.5 mm and the outer nozzle with OD of 1.00 mm. Nozzle length is 6.5cm. Maximum nozzle power is 20watts. Capacity of collection chamber is 250ml. Pumps can pump liquids up to

200ml/min. Solvent pump's connection goes to inner nozzle and water pump's connection goes to outer nozzle. Different stabilizers such as surfactants, polymers and electrolytes are added either in solvent or in water or in both. The apparatus used at New Jersey Institute of Technology is shown schematically in Figure 3.6.

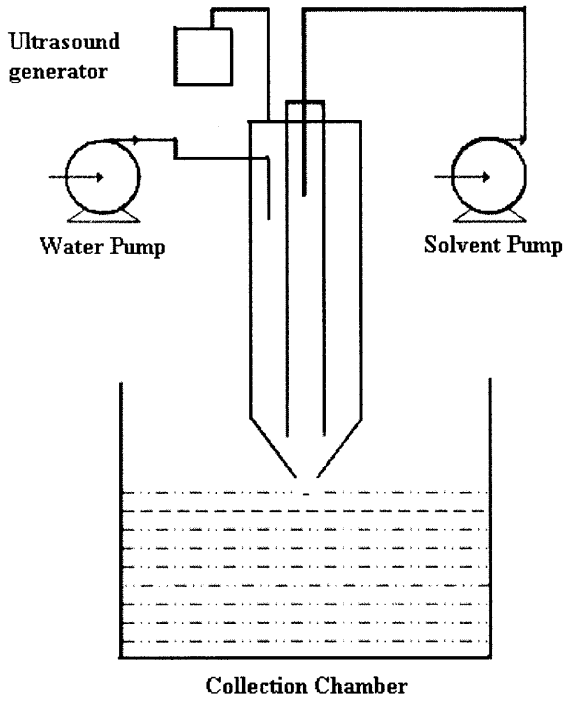


Figure 3.6 Schematic diagram for Liquid Antisolvent method with ultrasonic nozzle.

3.2.1.2 Precipitation of Ultrafine Particles using Subcritical CO_2. In this process, CO_2 acts as a co solvent for the formation of nano sized particles. Nano particles are formed due to large temperature drop caused by CO_2 expansion from working pressure to atmospheric pressure. Due to large drop in temperatures, solubility of the drug in the solution decreases and eventually drug particles are precipitated in the collection chamber, which is at atmospheric pressure. This is very simple technique. There are only

four steps involved for the production of nano particles. A simple flow diagram of the process is shown below.

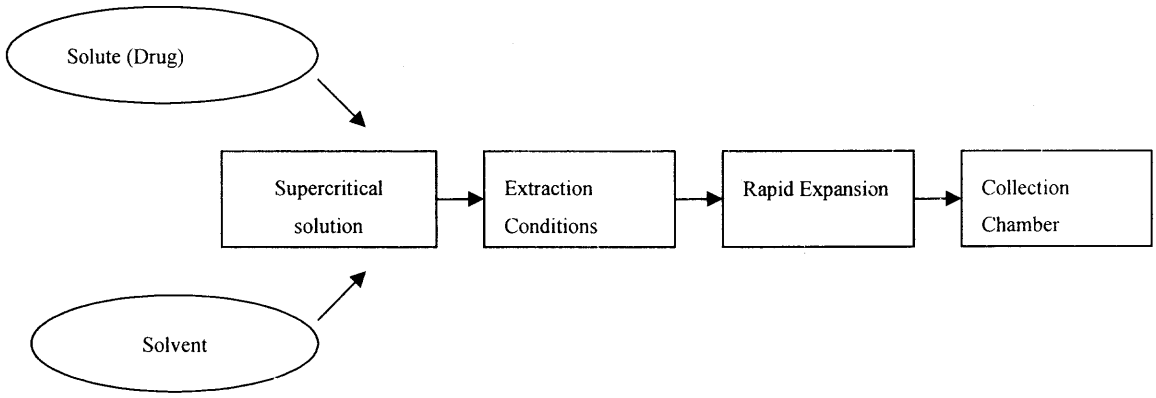


Figure 3.7 Flow diagram of precipitation of ultrafine particles using subcritical CO_2 Process.

The first step in this process is the preparation of the supercritical solution. The solution consists of supercritical solvent and a solute (drug / API). A liquefied solvent, at conditions higher than Tc and Pc, is pumped from the cylinder to an extraction chamber. The dissolution of solute in the supercritical solvent takes place in the extraction chamber. The dissolution properties of the solute depend on the extraction conditions (temperature and pressure) as well as the chemical characteristics of the solute. Therefore, it is essential to determine the solute solubility in supercritical solution through experiments or literature review. Required apparatus includes compressed CO_2 cylinder, extraction chamber, expansion chamber and nozzle. Both expansion and extraction chambers should be high-pressure vessels. Detailed procedure of conducting experiment is described below. The apparatus used at New Jersey Institute of Technology is shown in Figure 3.8.

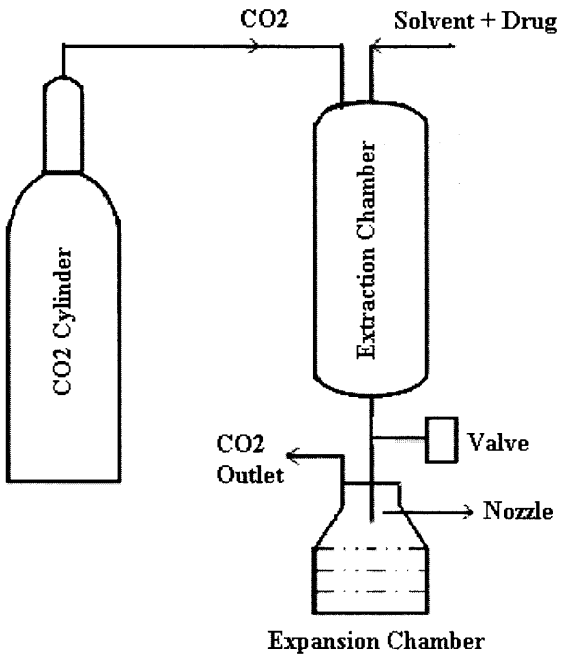


Figure 3.8 Schematic diagram for process using subcritical CO2 for precipitation of ultrafine particles.

Drug is dissolved in solvent and then fed to extraction chamber. Surfactant is added to the solvent if required. Extraction chamber is closed and filled with supercritical CO_2 at desired pressure. Allow some time to achieve equilibrium between the CO_2 and solvent. Depressurize the solution by opening the valve. Expansion chamber is filled with water. After depressurization, CO_2 evaporates from expansion chamber.

3.2.2 Characterization Techniques

Characterization of the particles precipitated using the above-described processes has been performed using scanning electron microscopy and Light scattering technique.

3.2.2.1 Scanning Electron Microscopy (SEM). SEM is used to observe the morphology of the particles. Scanning Electron Microscopy (SEM) is a very useful tool

for characterization of ultrafine particles. Images were taken using a Scanning Electron Microscopy (LEO 1530VP (FESEM-EDS) / Zyvex Nanomanipulator System) at an accelerating voltage ranging from 5 to 10 KV. The sample was deposited on a silicon chip fixed on aluminum stub. Liquid solution obtained from the experiment was sonicated for 30 minutes and few drops from this solution are deposited on the stub to analyze on SEM. The stub was then placed under vacuum in order to remove the moisture from the sample. Stubs must be completely dry in order to analyze them under SEM. SEM micrographs from different regions of the stubs were obtained.

Multiple images for each experiment were processed in order to get a proper representation of the particles. Several results obtained using SEM was compared with light scattering (where available) results and were found to be significantly similar.

3.2.2.2 Beckman Coulter - LS 230. Light scattering (Beckman Coulter LS 230) is the most sophisticated particle size analyzer with 132 optical detectors. The LS uses reverse Fourier lens, which enables it to optimize light scattering across the widest dynamic range in a single scan. It is used to measure hydrodynamic diameter of aqueous solution of drug and milli-Q water. Polarization Intensity Differential Scattering (PIDS) was also performed using Fraunhofer diffraction model. Range of particle size that can be analyzed is 0.04 μm to 2000 μm. Samples were first sonicated for 30 minutes and then used for analyzing.

CHAPTER 4

RESULTS AND DISCUSSION

Experiments were conducted to study the effect of operating parameters on the particle size, particle size distribution and morphology for Liquid Antisolvent process by using ultrasonic nozzle. Experiments are conducted by varying drug concentration, nozzle power, solution and solvent flowrate. Surfactants and polymers are added to water to stabilize and control the particle size. The following sections describe effects of varying different parameters.

4.1 Initial Screening of Process Parameters

Table 4.1 Effect of Operating Parameters and Surfactant

Exp	Power (watts)	Water Flowrate (ml/min)	T-80 (CMC)	Bath sonication	Mean particle size (μm)	SD
1	0	0	0	No	23.18	9.85
2	0	0	5	No	14.88	7.89
3	10	0	5	No	13.26	5.27
4	10	0	5	Yes	11.47	4.32
5	10	2	5	Yes	11.09	2.14

Experiment 1, is a simple liquid antisolvent process in which solution of the drug and the solvent is added to an antisolvent to precipitate the drug particles. Precipitated particles are about 23 microns size. In order to study the effect of surfactant, experiment 2 was

conducted by adding the Tween 80 (5 CMC) in the water used in collection chamber. At these experimental conditions, mean particle size of precipitated particles was around 14.9 microns. Addition of surfactant reduces the surface tension, increases the nucleation rate and also reduces the agglomeration. In order to study the effect of atomization, experiment 3 was conducted by applying power to nozzle. When the atomized droplets of solution came in contact with antisolvent, drug particles were precipitated. The particle size obtained is 13.26 microns. Application of ultrasonic nozzle atomizes the droplets and hence reduces the particle size.

The precipitated particles were collected in water in a collection chamber. As Griseofulvin is hydrophobic, the precipitated particles agglomerate in water. Therefore, in experiment 4 particles were collected in sonicated water using a bath sonicator. Particles produced were smaller than the particles formed from previous experiments due to the prevention of agglomeration. In experiment 5 both drug solution and water were pumped through a concentric nozzle vibrating at the tip. Vibrations due to ultrasound enhance the micro mixing of antisolvent and solution streams and increase the nucleation rate. This further decreases the particle size.

As it is seen that the process parameters affect the particle size and its distribution, it was therefore decided to generate DOE to identify important process parameters. Next section describes the DOE for liquid antisolvent experiments with ultrasonic concentric nozzle.

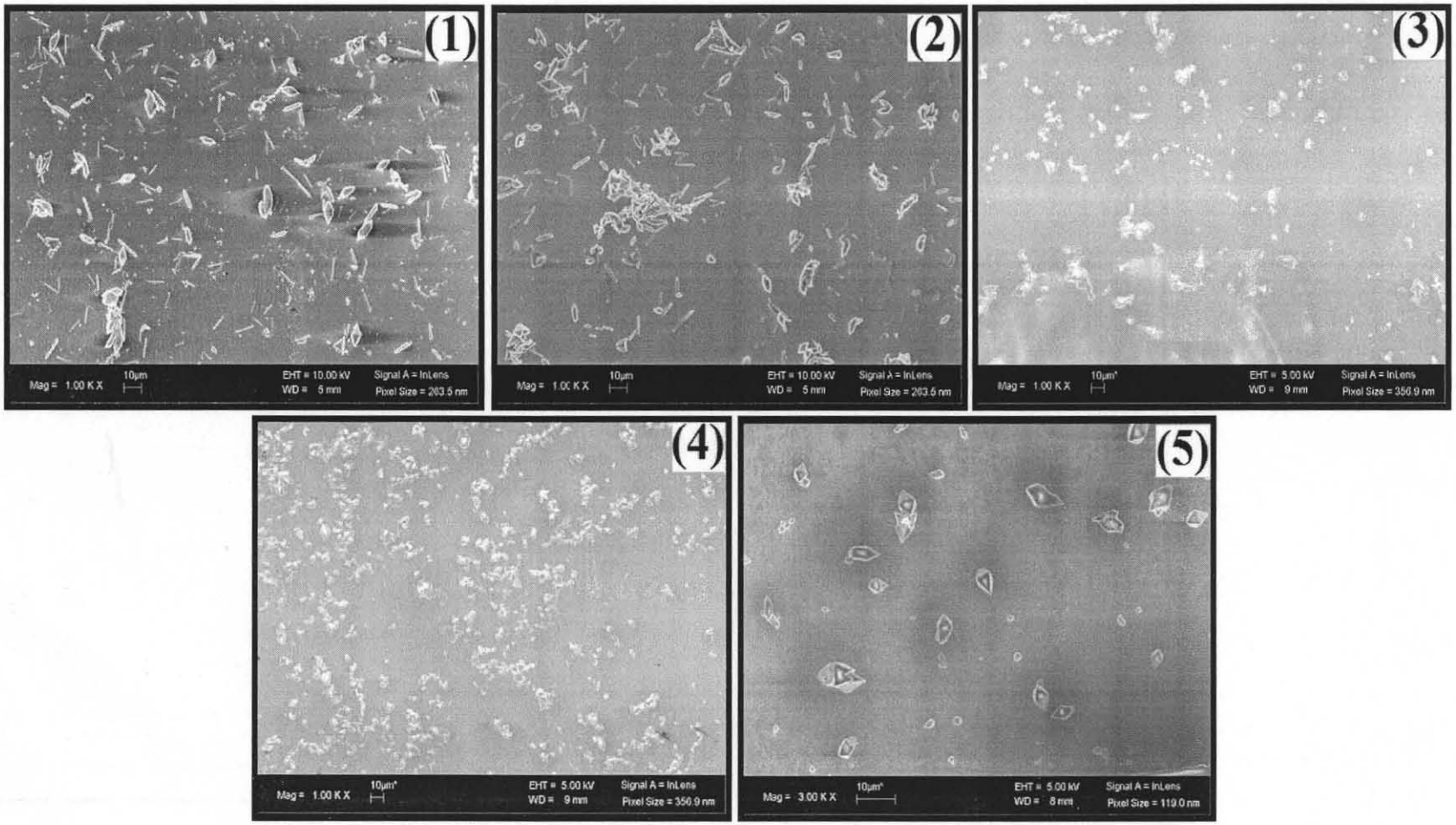


Figure 4.1 SEM micrographs for the experiments shown in Table 4.1.

4.2 Full Factorial Experiment by using DOE

Full factorial experiments were designed by using Minitab software. For full factorial design, three factors such as, concentration of the drug in the solvent, power applied to the nozzle and flowrate of antisolvent were considered. In this design, three levels were considered for each factor. Values for concentration were 0.01, 0.05 and 0.09, values of power were, 0, 5 and 10 and values for flowrate were, 0, 5 and 10. By following the DOE, total 27 experiments were conducted in a random order and the results are tabulated in Table 4.3. Procedure followed for conducting these experiments is described below.

The given amount of drug is dissolved in 10 ml of solvent. Power and water flow rate were adjusted as required. 200 ml of water along with surfactant Tween-80 was taken in the collection chamber. Concentration of Tween-80 was 5 CMC. For all these experiments, flowrate of drug solution was fixed at 2 ml/min. After each experiment, collected samples were analyzed using light scattering and SEM.

Table 4.2 Analysis Of Variance (ANOVA) Table

Source	DF	Seq SS	Adj SS	Adj MS	F	P
C	2	1111.118	1111.118	555.559	13.62	0.032
P	2	104.268	104.268	52.134	1.34	0.388
W	2	179.981	179.981	89.991	1.67	0.3
C*P	4	93.702	93.702	23.425	1.12	0.411
C*W	4	153.15	153.15	38.288	1.83	0.216
P*W	4	145.862	145.862	36.466	1.74	0.233
C*P*W	8	167.333	167.333	20.917	**	
Error	0	*	*	*		
Total	26	1955.415				

Table 4.3 Full Factorial Design to find out the Significant Factor

Exp	Drug weight (g)	Power (W)	Water flow (ml/min)	Water Collection (ml)	Size (microns)	S.D.
1	0.09	10	10	300	10.19	5.67
2	0.05	5	0	200	15.1	4.35
3	0.05	0	5	250	10.02	5.93
4	0.09	0	0	200	28.61	20.47
5	0.05	0	10	300	13.27	8.55
6	0.05	10	0	200	11.61	6.94
7	0.09	5	0	200	22.6	12.51
8	0.01	0	10	300	12.86	6.47
9	0.01	10	10	300	6.114	2.99
10	0.05	10	5	250	17.33	12.16
11	0.05	0	0	200	22.56	15.44
12	0.09	10	5	250	30.19	20.57
13	0.01	5	10	300	7.4	3.05
14	0.01	10	0	200	11.5	9.4
15	0.09	5	10	300	13.65	11.48
16	0.09	10	0	200	11.5	9.4
17	0.05	10	10	300	8.66	3.32
18	0.05	5	10	300	11.91	6.28
19	0.09	5	5	250	18.53	11.95
20	0.05	5	5	250	17.93	12.34
21	0.01	5	0	200	2.97	1.84
22	0.01	5	5	250	7.47	3.79
23	0.01	0	5	250	6.43	3.15
24	0.09	0	10	300	24.89	8.04
25	0.09	0	5	250	29.65	18.31
26	0.01	0	0	200	12.51	6.52
27	0.01	10	5	250	8.479	2.93

For this full factorial design experiment, ANOVA is conducted for the mean particle size. From the Table 4.2, it is clear that, concentration had significant effect on the particle size. Therefore, experiments were conducted by varying the concentration of Griseofulvin in acetone to study the concentration effect on the particle size, size distribution and morphology.

4.3 Effect of Concentration on the Particle Size

Table 4.4 Effect of Concentration on the Particle Size

Exp	Conc. (gm/ml)	Power (watts)	Mean (μm)	Median	Mode	S.D	D10	D50	D90
1	0.005	10	10.2	8.8	10.3	5.7	4	8.8	19.2
2	0.01	10	7.6	6.2	7.8	5.5	2.2	6.2	15
3	0.015	10	8.08	7.15	9.37	5.01	2.57	7.15	14.7
4	0.02	10	8.29	7.02	9.37	5.76	2.29	7.02	15.7
5	0.025	10	9.03	7.53	9.89	6.45	2.34	7.53	17.6
6	0.03	10	9.64	7.98	10.3	7.01	2.51	7.98	18.7
7	0.035	10	12.1	9.88	11.3	8.52	3.45	9.88	24.3
8	0.04	10	13.9	11.5	12.4	9.28	4.38	11.5	27.1

Table 4.4 and Figure 4.2 show the effect of concentration on the particle sizes and size distribution. It is observed that needle like bipyramidal particles were precipitated at all concentrations.

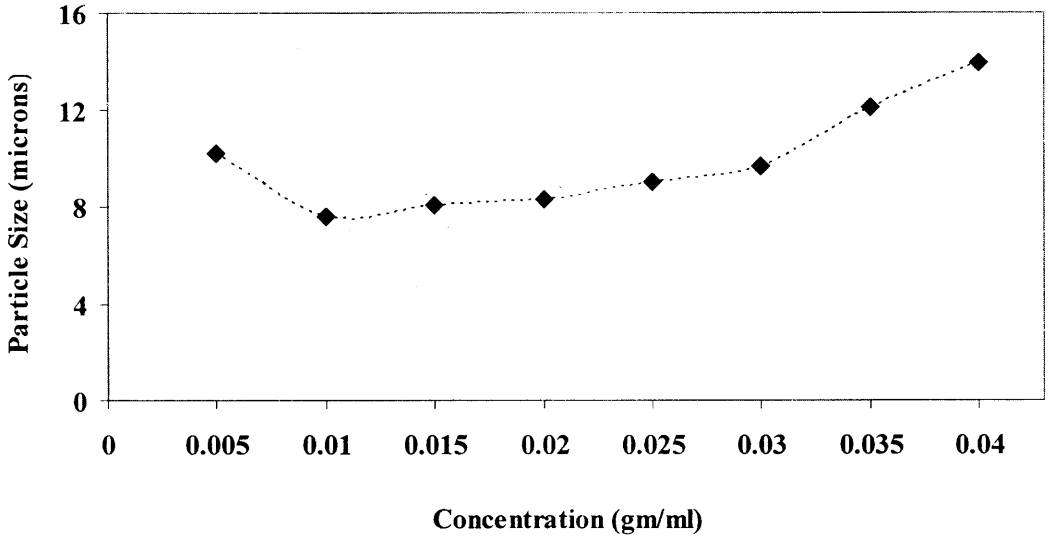


Figure 4.2 Effect of concentration on the particle size.

From experiments 2 to 8, particle size increased with increasing concentration of the drug. At lower concentration like 0.005 gm/ml, particle size was smaller than at other concentrations. The particle size goes through the minimum with change in concentration. At higher concentrations, there will be higher driving force for particle growth and increase in agglomeration increases the particle size. At lower concentration, there will be low supersaturation and lower nucleation rate increases the particle size.

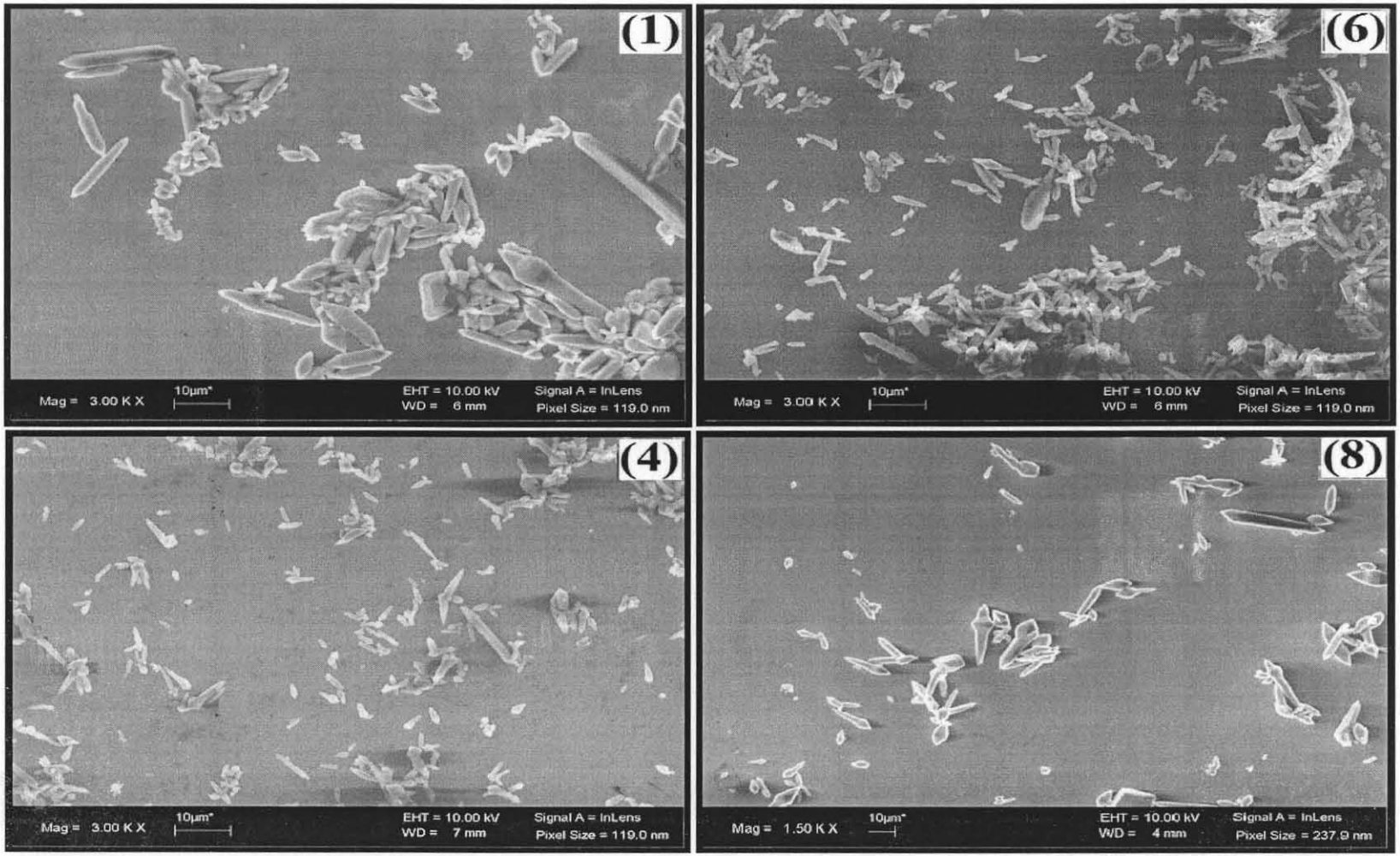


Figure 4.3 SEM micro graphs for the experiments 1, 4, 6 and 8 shown in Table 4.4.

4.4 Effect of Surfactant on the Particle Size

Surfactants reduce the surface tension of solution and there by increase the nucleation rate of precipitating solute. Surfactants stabilize the particle size by their specific adsorption on to the particle surface. Experiments have been carried out by adding surfactant in antisolvent phase and in organic phase separately.

4.4.1 Surfactant in Antisolvent Phase

Table 4.5 Effect of Surfactant (0.7 CMC) on the Particle Size when Surfactant is added in the Water

Exp.	Conc. (gm/ml)	Surfactant	Power (watts)	Mean (μm)	SD	D10	D50	D90
1	0.01	Tween 80	10	10.2	7.94	3.2	7.63	21.7
2	0.01	Poloxamer 188	10	8.67	5.96	3	7.05	16.4
3	0.01	SDS	10	6.37	4.64	2.2	5.07	12

Table 4.5 and Figure 4.5 show the effect of surfactant on particle size and particle size distribution. It can be seen that anionic surfactant SDS can control particle size and particle size distribution better than other surfactants. Adsorptions of anionic molecules of SDS on the particle surface develop negative charge on the surface of the particles and hence effectively control the agglomeration.

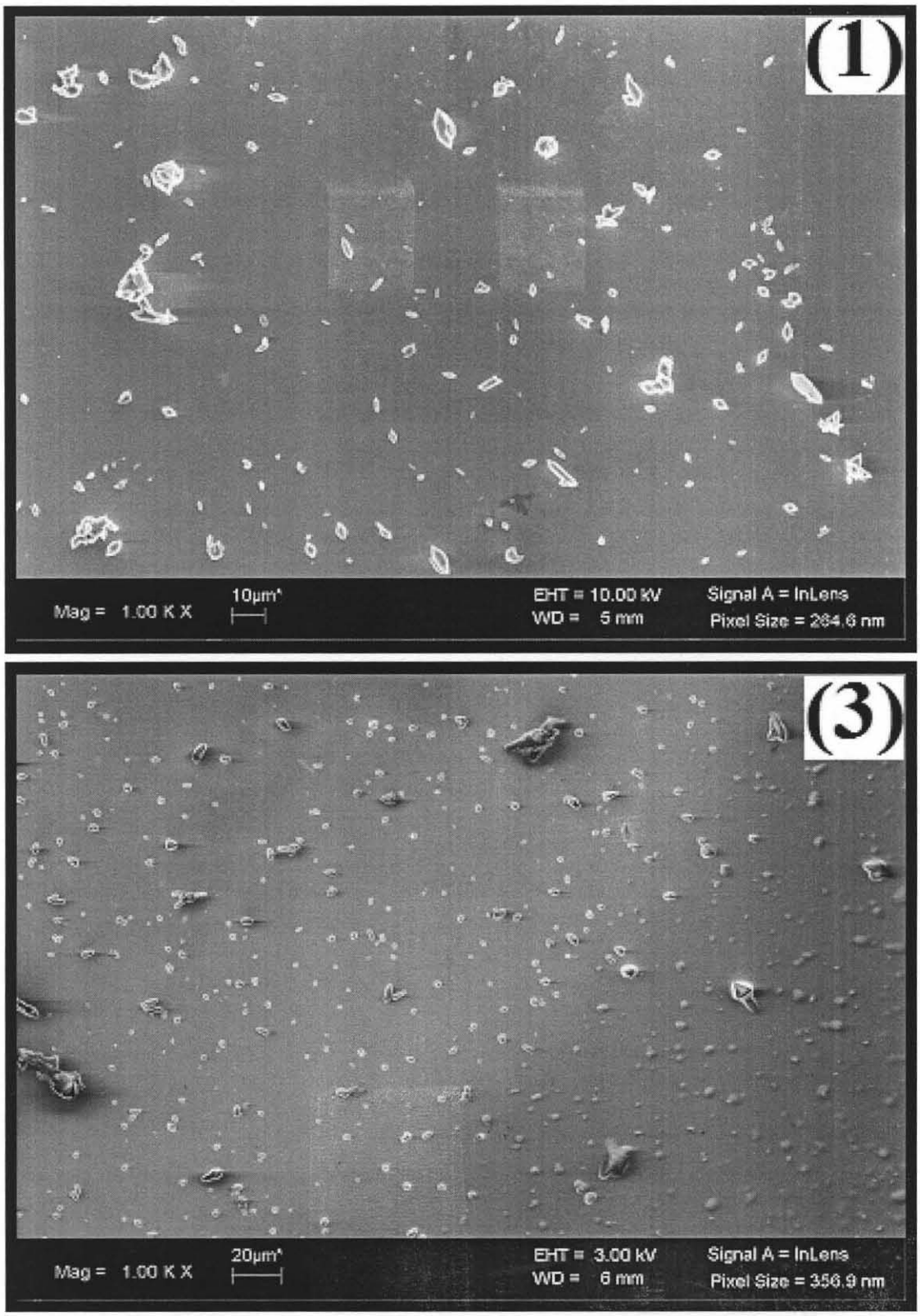


Figure 4.6 SEM micrographs for the experiments 1 and 3 shown in Table 4.6.

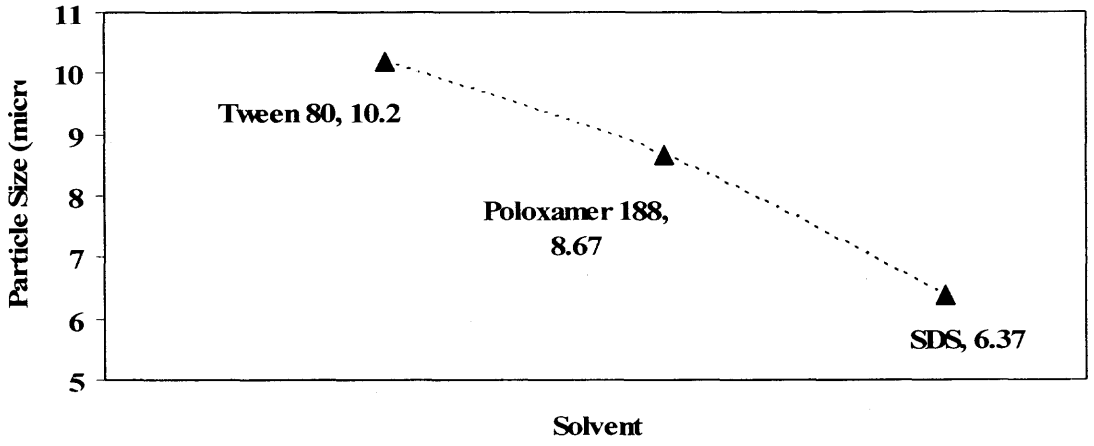


Figure 4.5 Effect of surfactant on the particle size.

4.4.2 Surfactant in Organic Solution Phase

Table 4.6 Effect of Surfactant (0.7 CMC) on the Particle Size when Surfactant added in the Solvent

Exp	Drug Conc. (gm/ml)	Surfactant	Power (watts)	Mean (μm)	SD	D10	D50	D90
1	0.01	Tween 80	10	10.5	7.17	3.6	8.48	20.2
2	0.01	SDS	10	*	*	*	*	*
3	0.01	Poloxamer 188	10	9.14	6.67	3.4	7.23	16.9

SDS cannot be dissolved in acetone and hence the data is not reported for such a condition. Comparison of Tables 4.5 and 4.6 shows that there is no much difference between the particle size with change in location of the surfactant.

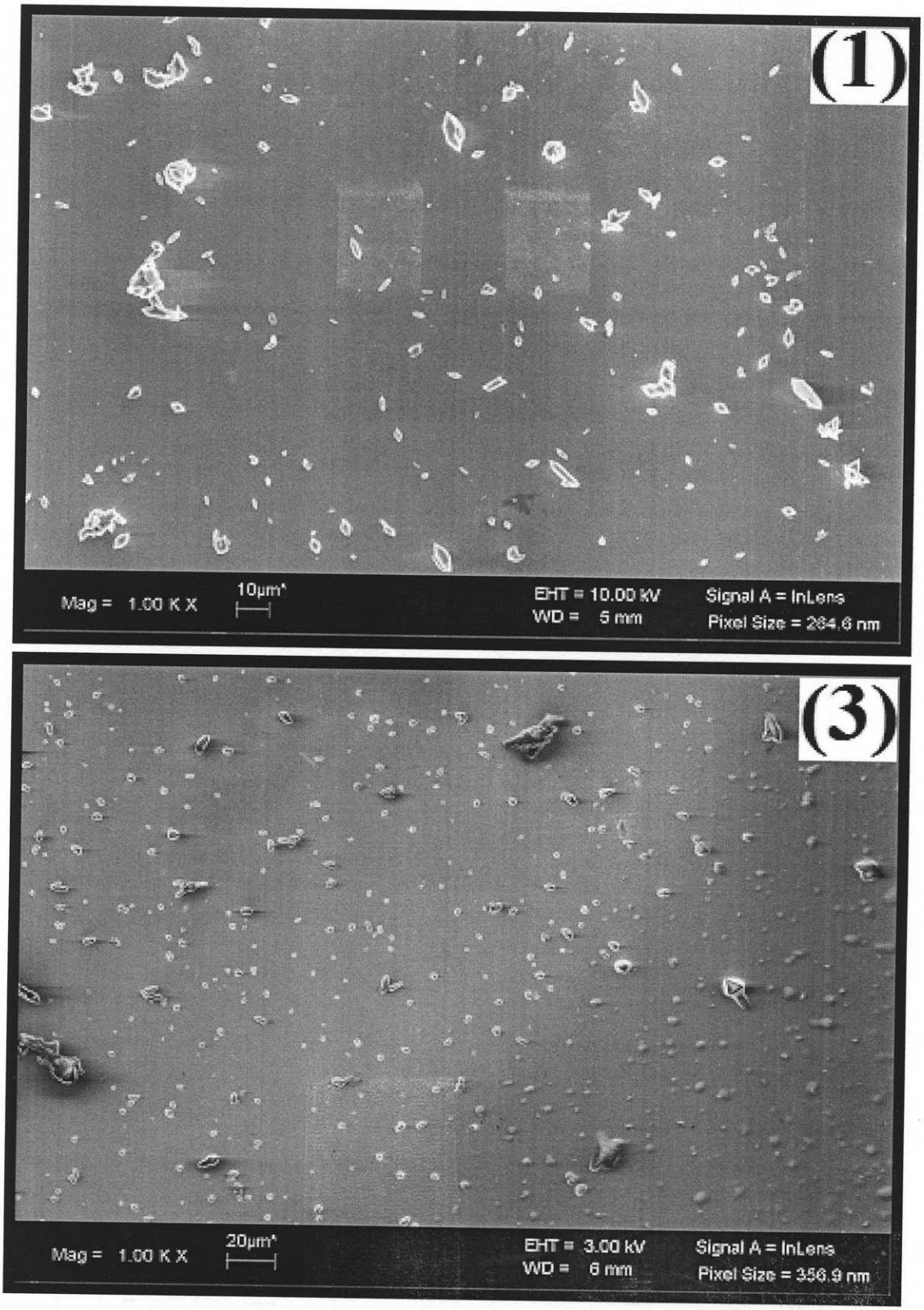


Figure 4.6 SEM micrographs for the experiments 1 and 3 shown in Table 4.6.

4.5 Effect of Polymer on the Particle Size

Table 4.7 Effect of Polymer on the Particle Size

Exp	Polymer weight. (gm)	Polymer	Power (watts)	Mean (μm)	SD	D10	D50	D90
1	0.01	HPMC	10	5.51	3.47	2.01	5.23	6.91
2	0.05	HPMC	10	9.47	7.95	3	6.84	21.5
3	0.1	HPMC	10	10.6	7.68	3.5	8.38	20.6
4	0.01	PVP	10	7.5	5.66	3.72	6.51	12.92
5	0.05	PVP	10	14.63	11.1	3.59	11.2	33.15
6	0.1	PVP	10	15.07	9.68	7.56	17.15	32.5
7	0.01	Polymer JR	10	6.24	3.29	2.6	5.55	11
8	0.05	Polymer JR	10	6.37	4.08	2.4	5.29	11.8
9	0.1	Polymer JR	10	7.91	5.71	2.6	6.4	14.8

Table 4.7 and Figure 4.7 shows that Polymer JR can control the particle growth better than HPMC and PVP. PVP has only one carboxyl group and can not form hydrogen bonds with the functional groups on Griseofulvin and hence is a poor candidate for the control of particle growth and hence the agglomeration and particle size. Cellulosic Polymers like HPMC and Polymer JR perform better than PVP as they have lots of hydroxyl group present in a molecule and can form hydrogen bonds.

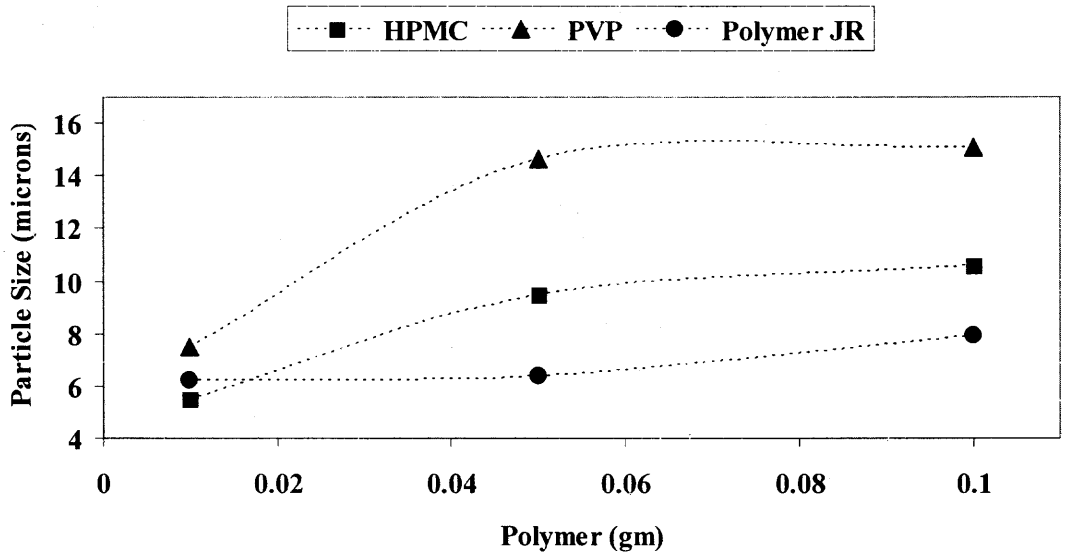


Figure 4.7 Effect of polymer on the particle size.

4.6 Effect of Polymer and Surfactant Combination on the Particle Size

Table 4.8 Effect of Polymer and Surfactant Combination on the Particle Size

Exp	Drug Conc. (gm/ml)	Polymer + Surfactant	Power (watts)	Mean (μm)	SD	D10	D50	D90
1	0.01	Polymer JR+SDS	10	8.209	5.96	2.65	6.53	16
2	0.01	HPMC+SDS	10	7.91	6.85	2.69	6.56	13.71
3	0.01	PVP+SDS	10	6.89	5.78	3.78	6.01	14.8

In order to study the effect of polymer and surfactant combination, 0.01 gm of SDS was added to 0.01gm of polymer and was used for conducting the experiments. Table 4.8 and Figure 4.9, show the effect of polymer and surfactant combination on the particle size and size distribution.

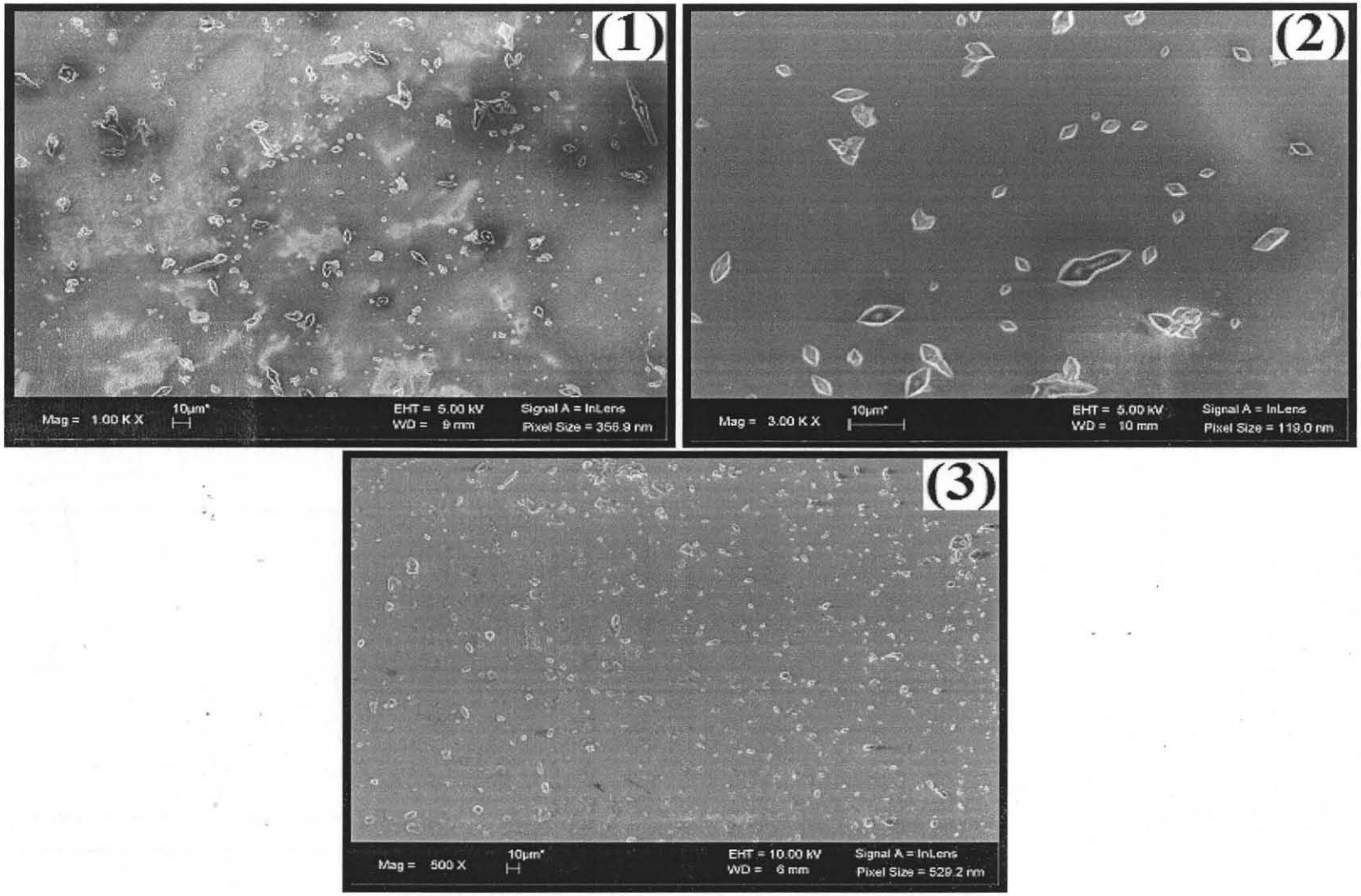


Figure 4.8 SEM micrographs for the experiments shown in Table 4.8.

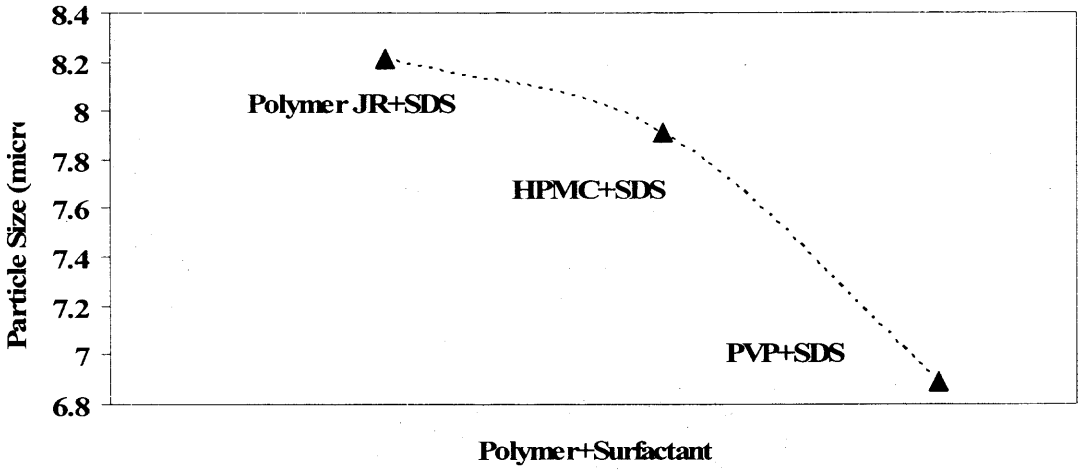


Figure 4.9 Effect of polymer and surfactant.

If polymer alone was used, HPMC was giving smaller particles than PVP. But by adding surfactant to the polymer, combination of PVP and SDS was giving better results than the combination of HPMC and SDS and Polymer JR and SDS. This is probably because the interaction between PVP and SDS is stronger than the interaction between SDS and HPMC or between SDS and Polymer JR. Because of this stronger interaction, SDS adsorption on PVP chains is more as compared to HPMC and Polymer JR and hence the PVP chains get straitened out more than HPMC or Polymer JR. Therefore PVP now can control the particle growth better than Polymer JR or HPMC.

4.7 Effect of Polymer, Surfactant and Electrolyte Combination on the Particle Size

Table 4.9 Effect of Polymer, Surfactant and Electrolyte Combination on the Particle Size

Exp	Drug Conc. (gm/ml)	Polymer+ Surfactant+ Electrolyte	Power (watts)	Mean (μm)	SD	D10	D50	D90
1	0.01	Polymer JR + SDS + NaCl	10	9.741	7.53	2.84	7.49	19.83
2	0.01	HPMC + SDS + NaCl	10	8.45	6.35	3.62	8.3	14.12
3	0.01	PVP+ SDS + NaCl	10	2.02	1.07	1.17	1.72	3.19

In order to study the effect of polymer, surfactant and electrolyte combination, 0.01 gm of SDS and 0.01 gm of NaCl was added to 0.01gm of polymer and was used for conducting the experiments. Table 4.9 and Figure 4.11 show the effect of polymer, surfactant and electrolyte combination on the particle size and size distribution. If polymer alone is used, HPMC is giving smaller particles than PVP. By adding surfactant to the polymer, combination of PVP and SDS is giving better results than combination of HPMC and SDS. By adding electrolyte to all polymer and surfactant combinations, PVP, SDS and NaCl combination is giving smaller particle size than other combinations. By adding NaCl, particle size is drastically reduced for the combination of PVP, SDS.

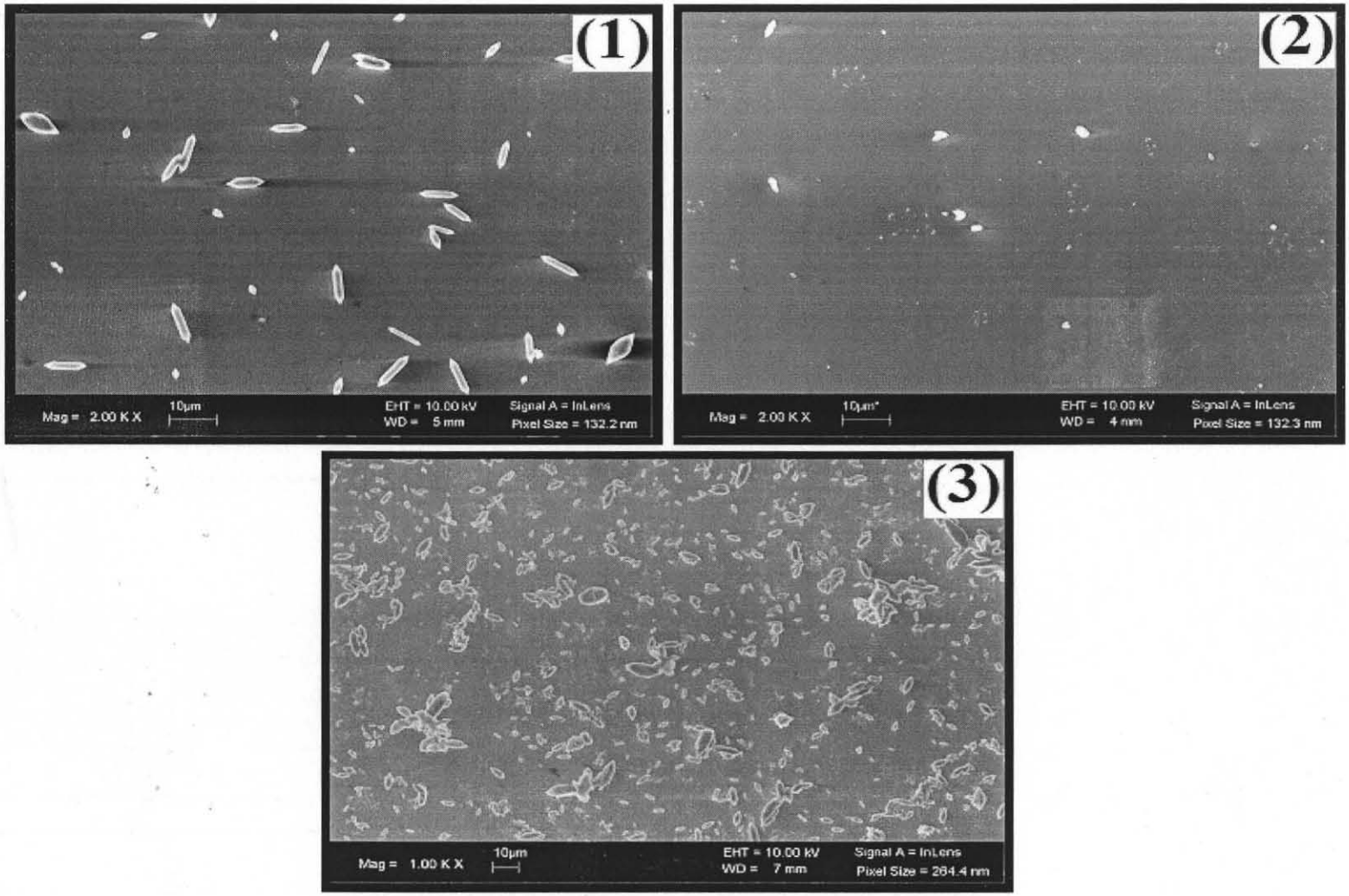


Figure 4.10 SEM micrographs for the experiments conducted in Table 4.9.

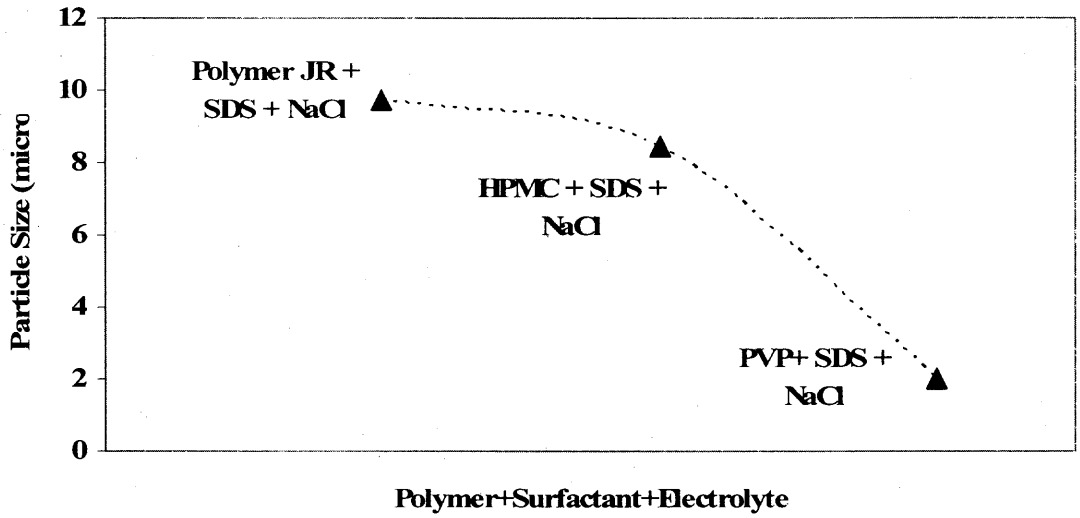


Figure 4.11 Effect of polymer, surfactant and electrolyte combination.

Addition of electrolyte NaCl to the solution enhances the interaction between PVP and SDS. Na^+ ions get adsorbed on the PVP chain and hence PVP behaves as a pseudo – poly cation. The negatively charged head group of SDS gets easily adsorbed on to the chain because of electrostatic interactions. This helps in straightening of an entangled polymer chain which now can better control the particle growth and reduce the particle size and size distribution.

Results tabulated in Table 4.10 were taken from Tables 4.7, 4.8 and 4.9, in order to study the effect of additives on particle size and size distribution. Experiments are conducted by adding polymer, combination of polymer and surfactant and combination of polymer, surfactant and electrolyte in antisolvent separately.

Table 4.10 Effect of Additives on the Particle Size

Exp	Conc. (gm/ml)	Additives	Power (watts)	Mean (μm)	SD	D10	D50	D90
1	0.01	HPMC	10	5.51	3.47	2.01	5.23	6.91
2	0.01	PVP	10	7.5	5.66	3.72	6.51	12.92
3	0.01	Polymer JR	10	6.24	3.29	2.6	5.55	11
4	0.01	HPMC+SDS	10	7.91	6.85	2.69	6.56	13.71
5	0.01	PVP+SDS	10	6.89	5.78	3.78	6.01	14.8
6	0.01	Polymer JR+SDS	10	8.209	5.96	2.65	6.53	16
7	0.01	HPMC+SDS+NaCl	10	8.45	6.35	3.62	8.3	14.12
8	0.01	PVP+SDS+NaCl	10	2.02	1.07	1.17	1.72	3.19
9	0.01	Polymer JR + SDS + NaCl	10	9.741	7.53	2.84	7.49	19.83

Table 4.10 and Figure 4.13 shows that, by adding SDS to HPMC, particle size increasing. Further addition of NaCl to HPMC and SDS, particle size is even bigger. In contrast, for PVP addition of SDS and NaCl reduces the particle size and distribution.

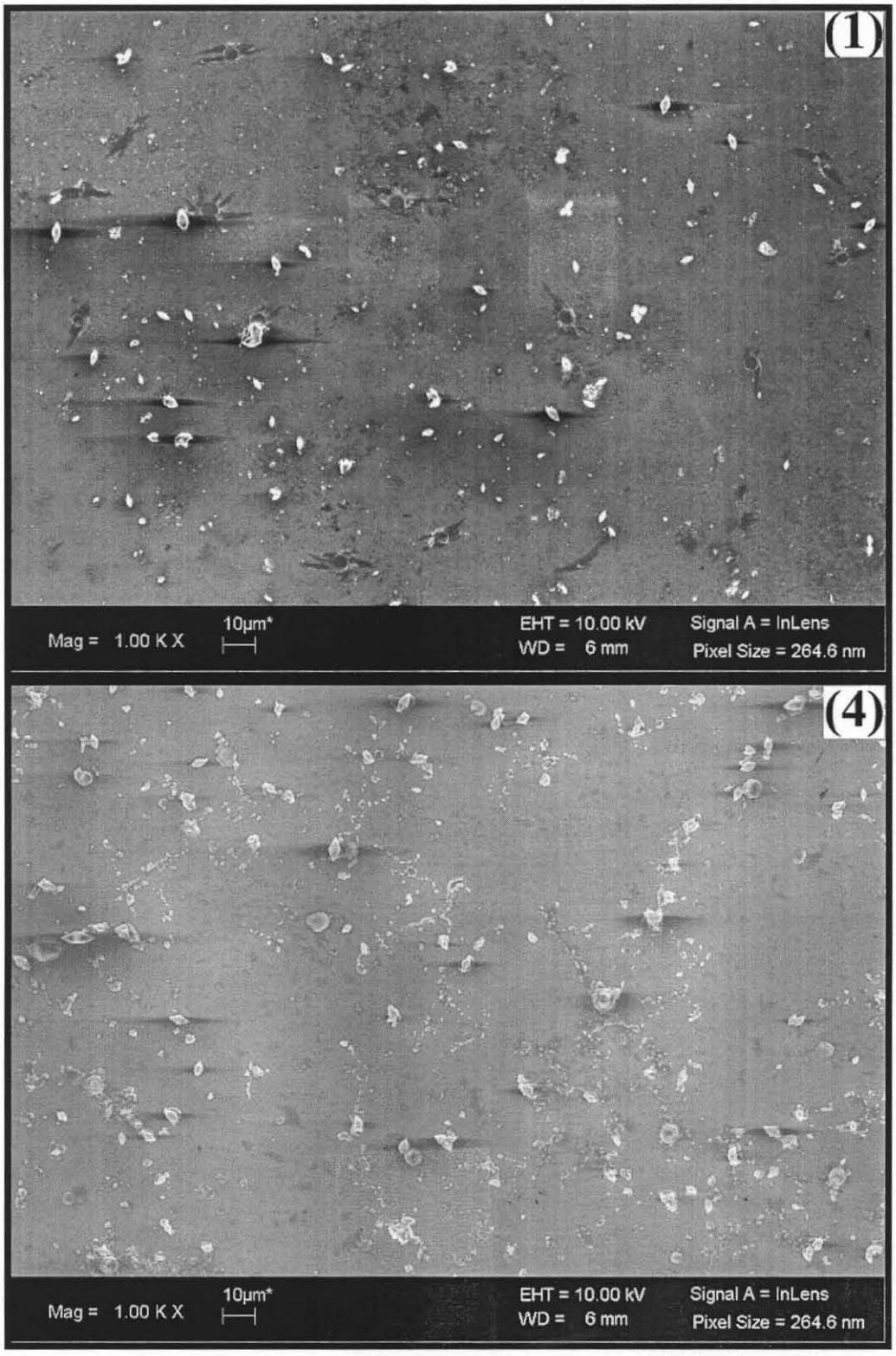


Figure 4.12 SEM micrographs for the experiments 1 and 4 shown in Table 4.10.

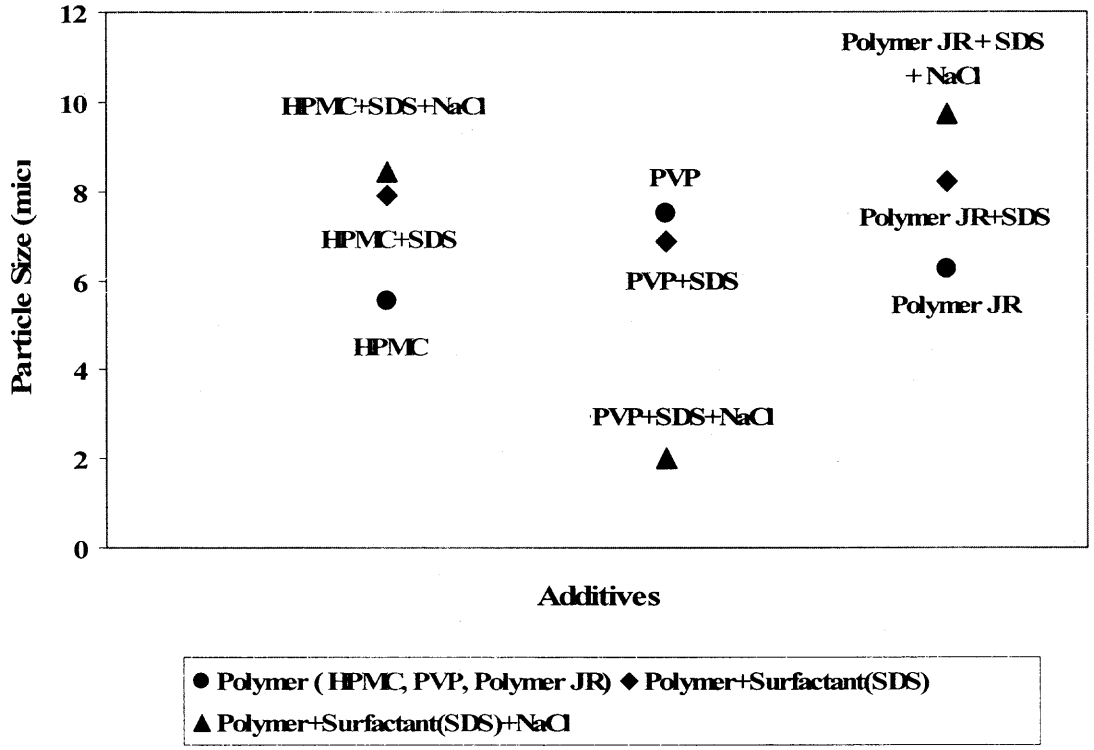


Figure 4.13 Graph showing effect of polymer, surfactant and electrolyte combinations.

The above experimental results show that if a polymer alone is added, HPMC is giving better results than PVP. Experiments 1, 2 and 3 in the Table 4.10, clearly indicate that HPMC is giving better results with no addition of surfactant and electrolyte. However, experiments 4, 5 and 6 show that PVP is giving better results with the addition of surfactant SDS and electrolyte NaCl. Comparing all the experimental results from Table 4.10, it is clear that combination of PVP, SDS and NaCl gives the narrowest particle size distribution.

From above experiments it was observed that, by using PVP it was possible to obtain smaller particles. To find out the size range of particles at saturated concentration of Griseofulvin in antisolvent, following experiments were conducted.

Table 4.11 Effect of Polymer, Surfactant and Electrolyte at Concentration 0.04 gm/ml

Exp	Conc. (g/ml)	Additives	Power (watts)	Mean (μm)	SD	D10	D50	D90
1	0.04	PVP	10	10.3	7.2	2.93	8.61	19.81
2	0.04	PVP+SDS	10	4.15	2.96	1.61	3.34	7.86
3	0.04	PVP+SDS+NaCl	10	3.75	2.69	1.52	2.82	6.88

If experiments 4, 5 and 6 in Table 4.10 and experiments in Table 4.11 are compared, with increase in drug concentration, particle size is increasing. In both tables 4.10 and 4.11, experiments done with PVP, SDS and NaCl, with increase in concentration there is not much increment in particle size. To avoid using more solvent, experiments can be conducted at higher concentrations if the obtained size is acceptable.

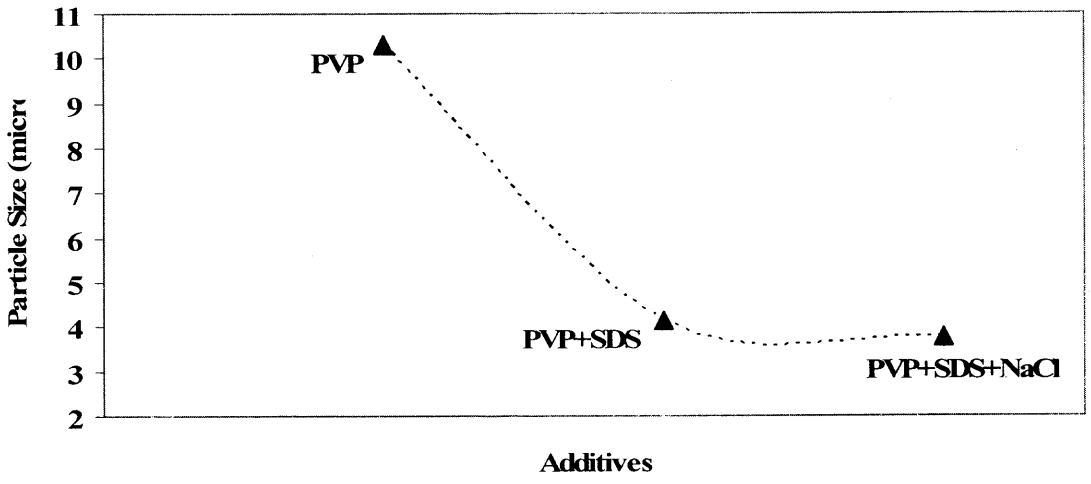


Figure 4.14 Effect of Polymer, Surfactant and Electrolyte at Concentration 0.04 gm/ml.

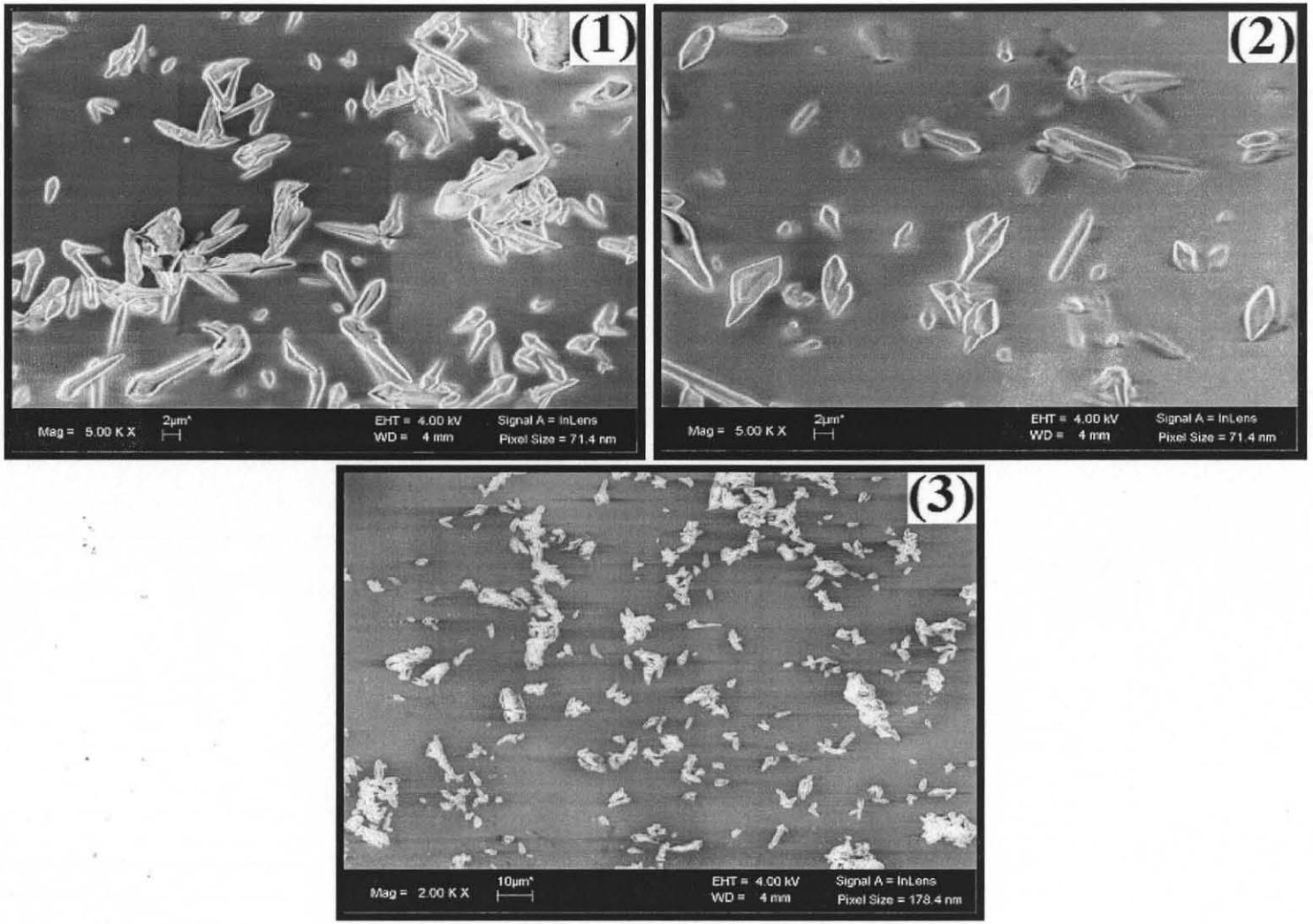


Figure 4.15 SEM micrographs for the experiments shown in Table 4.11.

Table 4.12 Concentration of Components in Aqueous Suspension

Component	gm	wt%
Drug	0.4	0.16
Solvent	7.85	3.05
water	249	96.77
HPMC	0.01	0.004
PVP	0.01	0.004
Polymer JR	0.01	0.004
Tween 80	0.01	0.004
SDS	0.01	0.004
Poloxamer 188	0.01	0.004
NaCl	0.01	0.004

4.8 Precipitation of Ultrafine Particles using Subcritical CO_2

In this method Griseofulvin is dissolved in organic solvent and the prepared organic solution is pressurized with CO_2. After allowing CO_2 and organic solution to equilibrate for 30 minutes, solution is depressurized through a valve in water containing stabilizers such as Tween 80. Depressurization of solution containing dissolved CO_2 causes evaporation of CO_2 from solution. Removal of latent heat of vaporization from the solution, reduces the solution temperature. This generates super saturation in solution due to reduction in equilibrium solid solubility in solvent induces nucleation and initiates the precipitation process. Presence of surfactant in water controls the particle growth and agglomeration by adsorbing on to the particle surface. Light scattering and SEM was used to characterize the precipitated particles. Effect of process parameters such as initial pressure, concentration and solvent characteristic have been studied on particle size and distribution.

4.8.1 Effect of Concentration on the Particle Size

Following experiments were conducted in order to study the effect of concentration, with different solvents, on the particle size. Experiments were conducted at different concentrations by maintaining all other conditions constant. From the results shown in Table 4.13 and figure 4.14, it was found that increasing drug concentration resulted in larger particles and broader size distribution. Same trend was observed in the particle size even with a different solvent.

Table 4.13 Effect of Concentration on the Particle Size

Exp	Pressure (bar)	Solvent	Temp (°C)	Conc. (gm/ml)	Size (μm)	SD
1	55	Acetone	26	0.001	5.37	3.2
2	55	Acetone	26	0.005	8.18	3.68
3	55	Acetone	26	0.01	9.1	5.32
4	55	Acetone	26	0.02	15.07	9.66
5	55	DCM	26	0.001	3.68	0.9
6	55	DCM	26	0.005	5.87	5.53
7	55	DCM	26	0.01	7.24	1.75
8	55	DCM	26	0.02	8.49	2.21

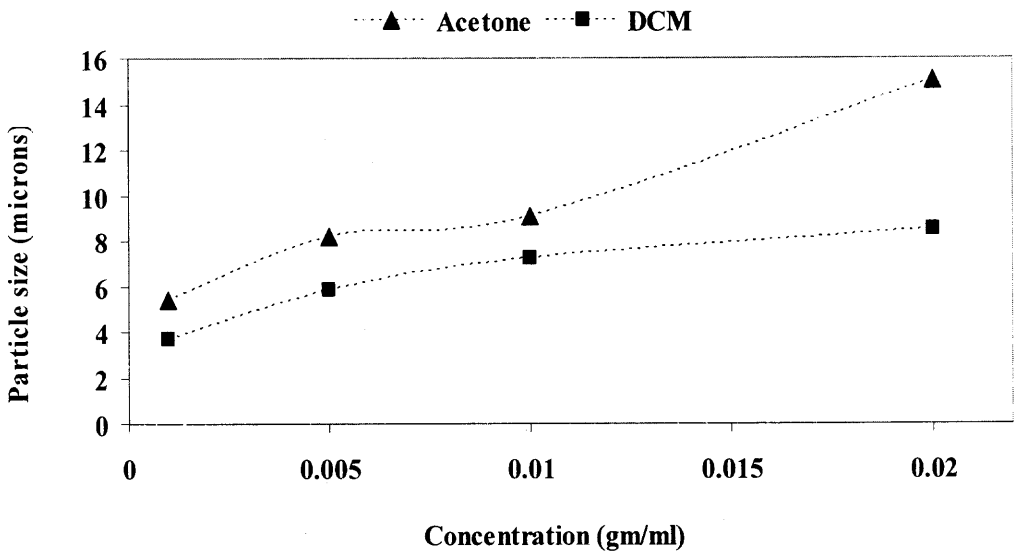


Figure 4.16 Effect of concentration on the particle size.

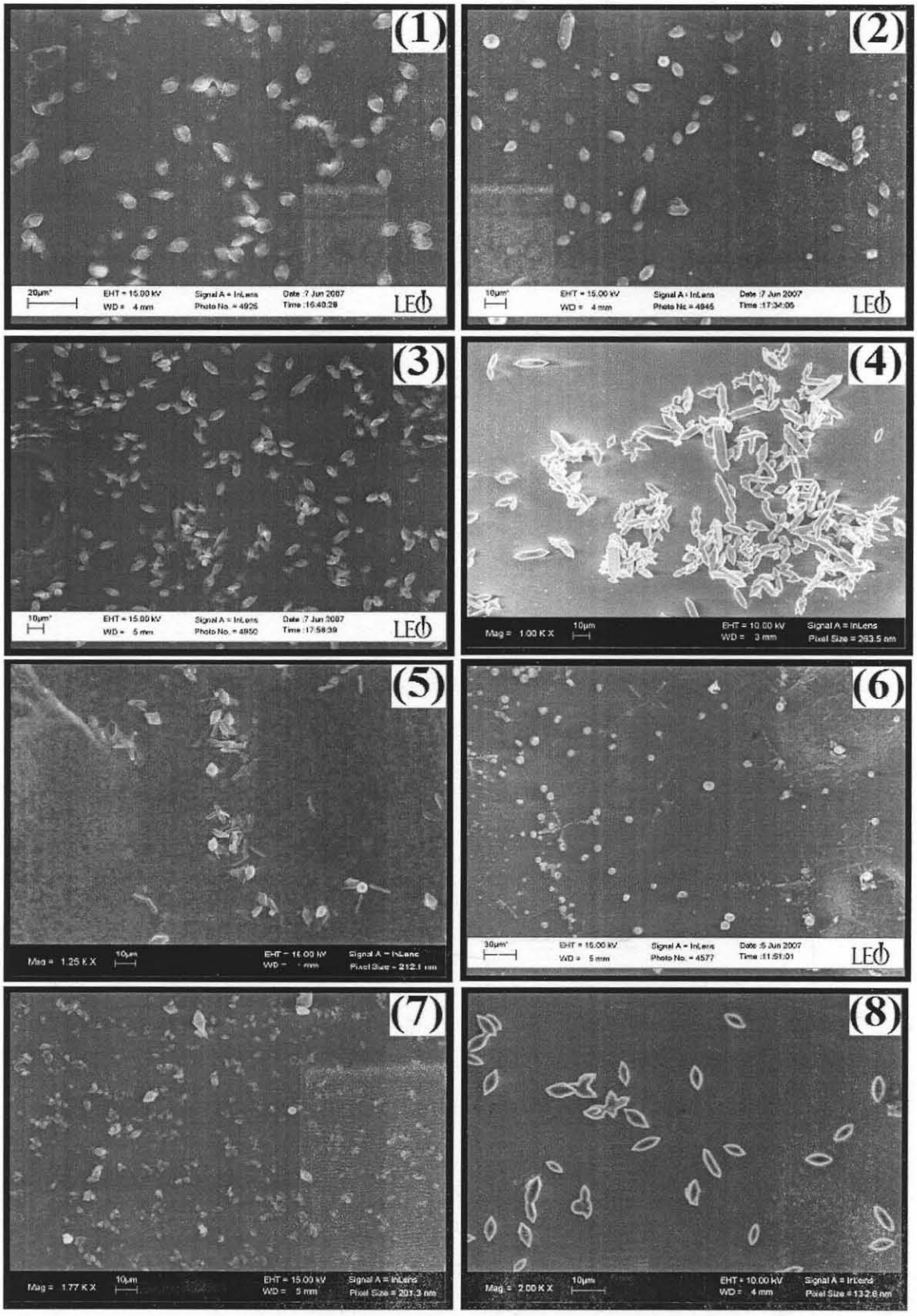


Figure 4.17 SEM micrographs for the experiments shown in Table 4.13.

From the above results, it was clear that lower concentrations can make particles with narrow size distribution. For this process, along with drug concentration, particle size also depends on operating parameters like pressure and the solvent chosen.

4.8.2 Effect of Solvent on the Particle Size

Table 4.14 Effect of Solvent on the Particle Size

Exp	Conc. (gm/ml)	Solvent	Size (μm)	SD
1	0.003	Acetone	7.62	5.05
2	0.003	DCM + Acetone	6.23	5.49
3	0.003	DCM	1.97	1.94

Solubility of Griseofulvin in DCM is higher than the Griseofulvin solubility in acetone. Solubility of CO_2 is higher in DCM than in acetone as DCM being non polar and acetone being polar aprotic, higher CO_2 dissolution causes more temperature drop and therefore higher supersaturation is generated when DCM is used as solvent. Higher supersaturation generates higher nucleation rate and hence particle size decreases.

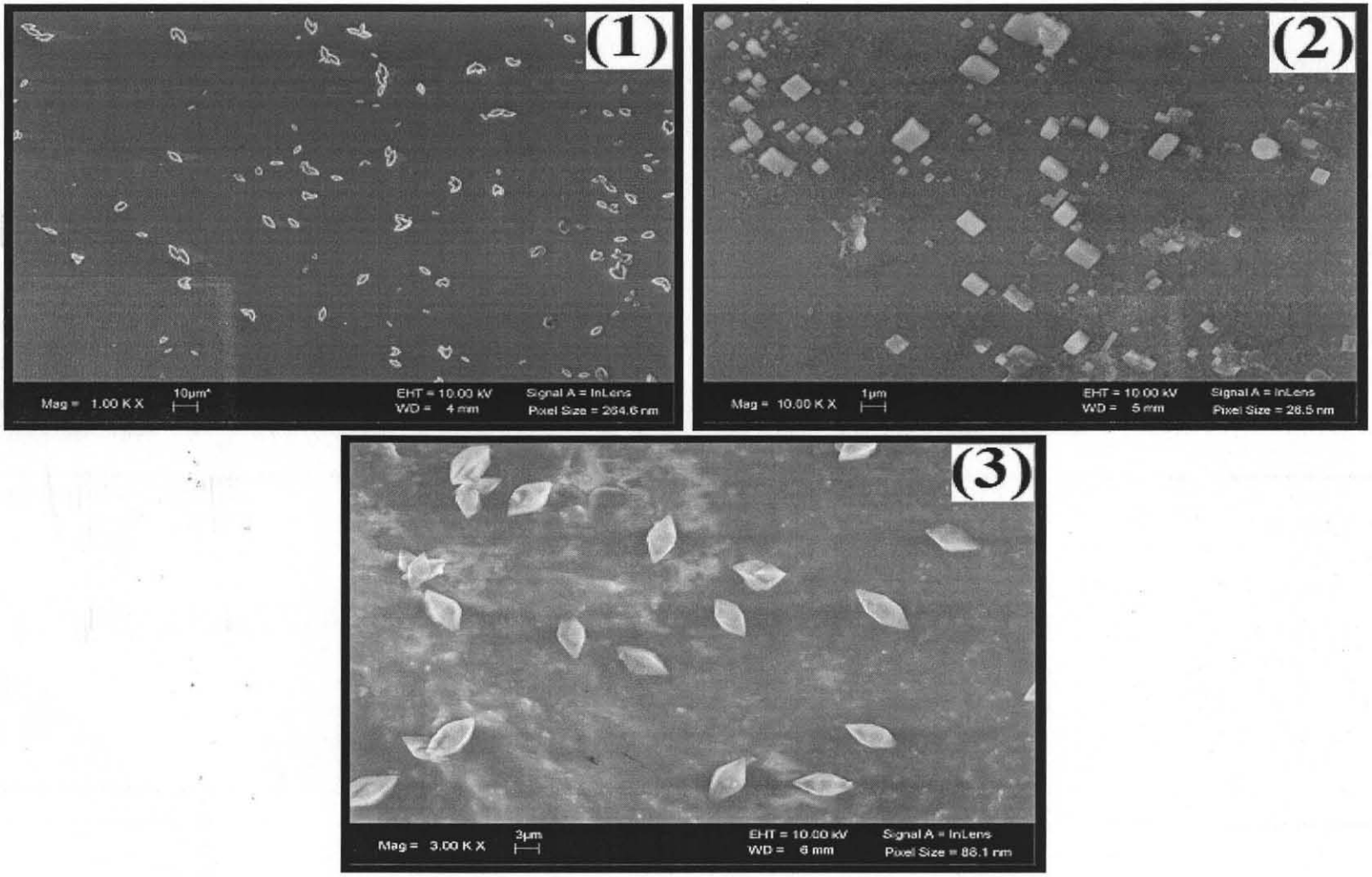


Figure 4.18 SEM micrographs for the experiments conducted in Table 4.14.

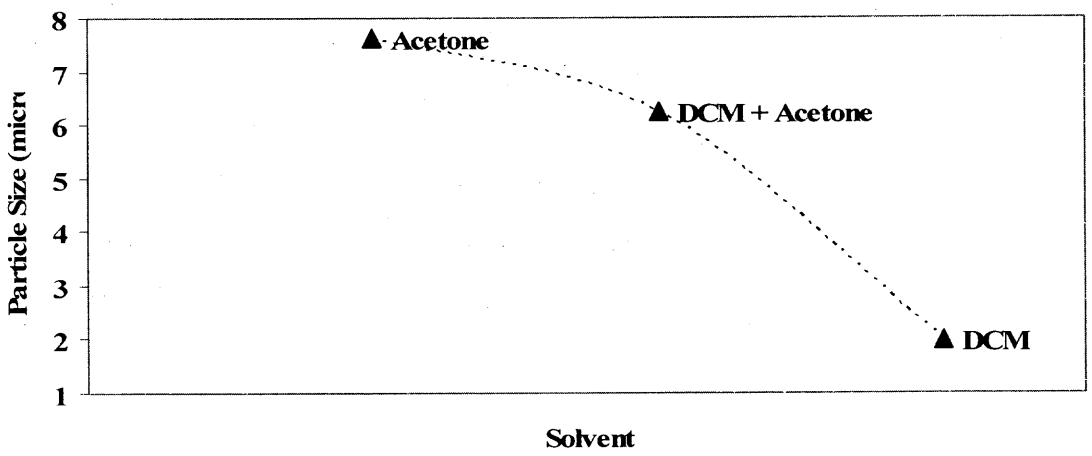


Figure 4.19 Effect of solvent on the particle size.

From the results shown in Table 4.14 and Figure 4.19, particles produced by Acetone were bigger than the particles produced by DCM. Mixture of Acetone and DCM also gave bigger particles compared to DCM. From the SEM micrographs shown in Figure 4.16, shape of the particle varies with the solvent. Diamond shaped particles were produced by using pure acetone and mixture of Acetone and DCM. Rectangular shaped particles were formed by using DCM.

4.8.3 Effect of Pressure on the Particle Size

The effect of initial pressure (50 - 70 bars) was explored for this process while keeping all the remaining parameters constant. Increase in pressure, increases the depressurization time and hence increases the agglomeration of particles during precipitation and hence increase the particle size.

Table 4.15 Effect of Pressure on the Particle Size

Exp	Pressure (bar)	Conc. (gm/ml)	Solvent	Size (μm)	SD
1	70	0.015	DCM	8.64	6.35
2	65	0.015	DCM	7.45	4.05
3	60	0.015	DCM	5	4.61
4	55	0.015	DCM	4.44	4.26
5	50	0.015	DCM	2.43	1.85

Results shown in Table 4.15 and Figure 4.20 indicate that an increase in initial pressure resulted in the increase in the particle size of Griseofulvin drug particles. The particle size distribution was also found to be widened with an increase in pressure. For this process smaller particles were produced by maintaining lower pressures.

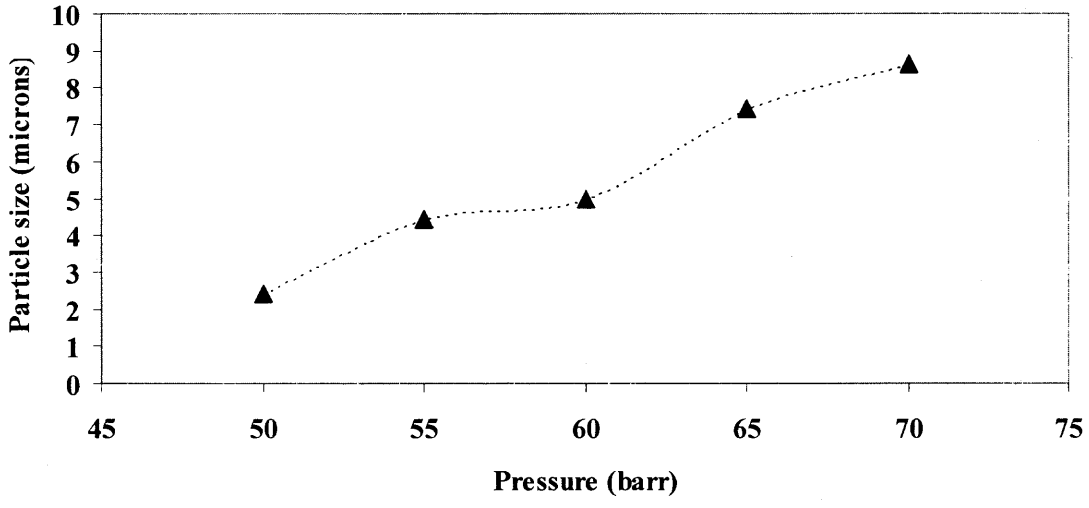


Figure 4.20 Effect of pressure on the particle size.

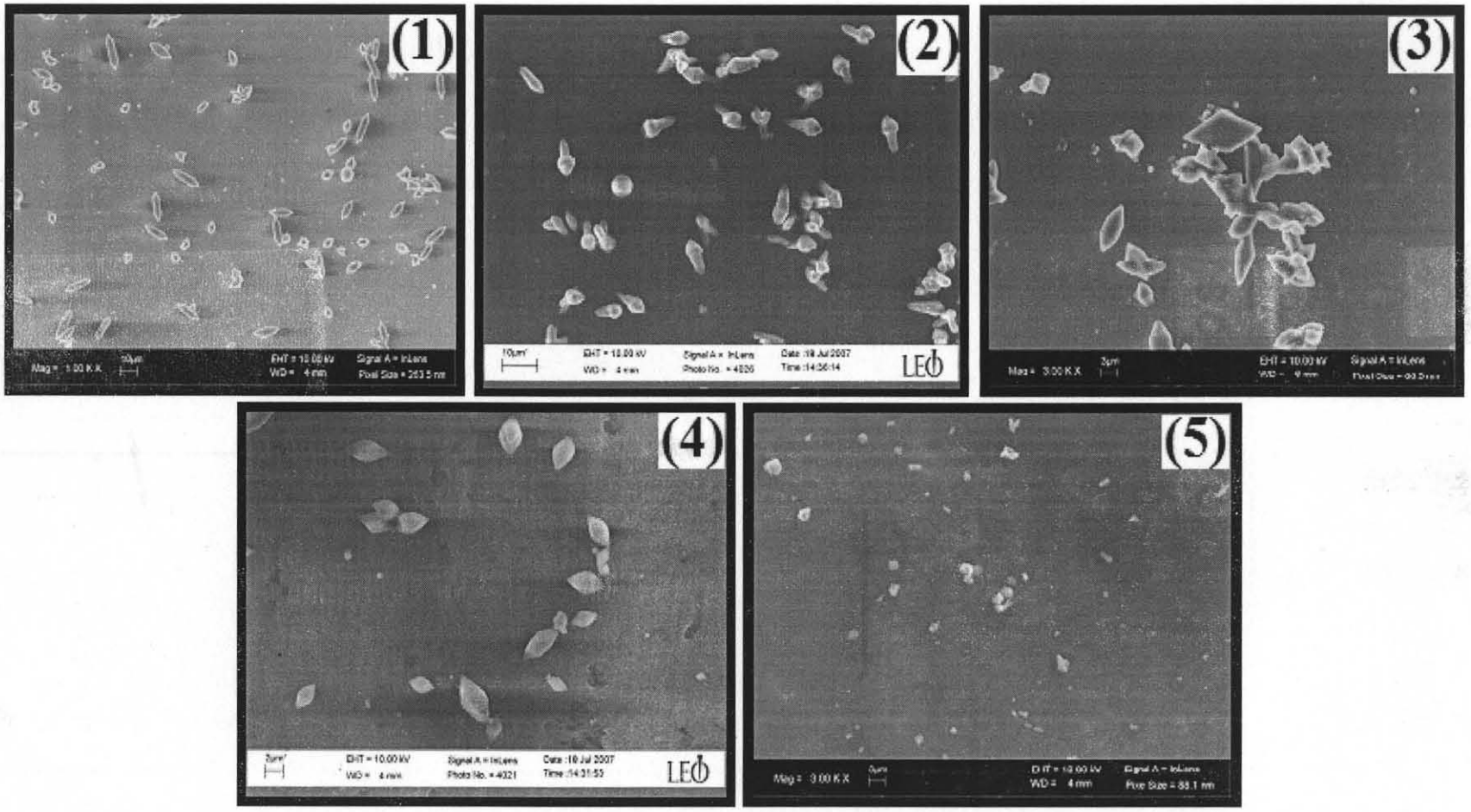


Figure 4.21 SEM micrographs for the experiments conducted in Table 4.15.

Table 4.16 Concentration of Components in Aqueous Suspension

Component	gm	wt% (If Acetone is used)	wt% (If DCM is used)
Drug	0.2	0.08	0.08
Acetone	7.85	3.03	-
DCM	13.255	-	5.01
water	249	96.15	94.11
Tween 80	2.12	0.82	0.80

CHAPTER 5

CONCLUSIONS AND RECOMMONDATIONS

This study focused on two processes for the production of fine particles namely Liquid Anti solvent using concentric ultrasonic nozzle and the other using subcritical CO_2. Various process parameters on particle size and size distribution were studied. Griseofulvin with size ranging from 10 - 2 microns was precipitated with a narrow particle size distribution. It was observed that the morphology of particles was always diamond shaped or tetragonal. Efforts were made to control the particle size using a combination of ultrasound and additives such as polymer and surfactants for Liquid antisolvent using ultrasonic nozzle. Use of ultrasound increases micromixing and surfactant and polymers decrease surface tension of solution. Enhanced micromixing and reduction in surface tension increases nucleation rate and reduces particle size. Further adsorption of polymers/surfactants and their complexes on the particle surface control the particle growth. Addition of electrolyte further enhances the interaction between polymers and surfactants and further narrows down the particle size distribution. For the process of particle precipitation using CO_2 it is observed that lower pressure, lower the initial solute concentration and non polar solvent decrease the particle size and distribution. Generation of higher supersaturation with controlled growth can precipitate ultrafine particles in this process.

It can be recommended that there should be a detailed study conducted on the mechanism of particle formation so as to be able to tune the particle size and size distribution by varying process parameters.

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